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THE NEWER KNOWLEDGE OF NUTRITION

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THE NEWER KNOWLEDGE OF NUTRITION

THE USE OF FOODS FOR THE PRESERVATION OF VITALITY AND HEALTH

BY

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To
DR. C. EIJKMAN
Professor of Hygiene
in the University of Utrecht,
Who First Produced Experimentally
a Disease of Dietary Origin,
THIS BOOK IS DEDICATED

PREFACE

The field of nutrition is attracting investigators in ever increasing numbers. To-day there are scores active in researches on the effect of diet where ten years ago there was one. The increase in the volume of technical papers is at least proportional. Since the second edition of this book appeared three years ago so many results of importance have appeared that it is now antiquated and a new edition is called for. This third edition is almost entirely re-written, only a portion of the earlier chapters being preserved. Re-writing was necessary in order to permit of the deletion of sufficient material to make room for an account of recent progress without increasing materially the size of the book.

The chapter on rickets has been expanded to cover the marked advances which three years have brought forth in an understanding of the manner in which bone growth can be modified by diets faulty in different respects. A chapter has been added in which have been gathered the principal contributions relating to the effect of diet on resistance to disease and the mechanisms of the processes involved insofar as information is available. A separate chapter is devoted to the relation of iodine deficiency to goiter. The properties of the strictly carnivorous diet have been further analyzed. The data relating to bios and the vitamin E (the X substance of Evans and Bishop) are included. The dietary habits of man in different parts of the world are described in greater detail than in the former edition, and the reasons for success or failure of nutrition of certain peoples are discussed. The chapter on the dietary properties of individual food-stuffs has been expanded to include recent advances.

It was not deemed worth while to include a table of the vitamin values of different foods, since there is great variation in different samples. There is no adequate procedure for estimating quantitatively any vitamin, so data of a general nature is all that can be presented without giving a false impression as to values. All existing tables are very misleading in this respect.

The objective of the authors was to make this a comprehensive

treatise on nutrition. The subject has been treated in an historical way, since this brings it before the reader in a better perspective than any other. Much of the experimental work is still too recent to make an intelligent criticism possible. A critical examination of special phases of the subject has been undertaken only where the special knowledge of the authors seemed to warrant it.

A bibliography sufficiently comprehensive to make it suitable for reference for those who desire to make use of original sources of information is included. It contains references to what were believed to be all the papers of importance up to December, 1924.

E. V. McCollum.
Nina Simmonds.

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THE NEWER KNOWLEDGE OF NUTRITION

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CHAPTER I

THE BEGINNING OF A NEW ERA IN NUTRITION STUDIES

In the first decade of the twentieth century there was a renewal of active study of the problems of nutrition by refined experimental methods. The field was then considered by those familiar with it as a well worked one, holding out little prospect of new and important discoveries. Yet the second decade of that century saw the greatest advances which have been made in our knowledge of the nutritive needs of the living body and of the specific properties of natural foods.

These results were the outcome of advancing knowledge of the chemistry of food-stuffs, especially of the proteins and the amino-acids into which they are digestible, and of a more rational type of animal experimentation than had hitherto prevailed. Progress has resulted in the past and will continue to come in the future from the judicious division of labor between the study of food problems by chemical methods and by animal experimentation. In this account of the present situation respecting our knowledge of foods and of nutrition it is desired to present to the reader an appreciation of the viewpoint of the investigator. It will be possible in this way to convey to him an appreciation of the line of reasoning by which the successive steps in the progress of the last few years have been attained. A brief historical account of the researches in this field will serve this purpose.

Components of Protoplasm.—A plant structure or an animal body is an exceedingly complex system of chemical substances. Many are far more complex in their structure than the most complicated machine. The first step in reaching an understanding of the chemistry of the living tissues must involve the

separation and study of the structural units of which they are composed. This was the field of activity of many organic and biological chemists during the nineteenth century. Through these studies it became established that the body of an animal or the tissues of a plant consist essentially of proteins, carbohydrates, fats and a series of related substances which are collectively known as lipins. Besides these the living tissues contain more or less water and a number of inorganic elements in molecular or ionic form. The fats and the simpler substances into which they can be converted in the process of saponification were exhaustively studied. The complex carbohydrates and the simpler sugars were discovered, and the manner in which they are related chemically was made clear. The proteins are the most complex of the food substances and were the most difficult to study chemically. Familiar examples of these are egg white, casein of milk, and the principal components of meat. The proteins are rich in nitrogen, the average content of this element being not far from 16 per cent. Certain proteins which occur in our plant and animal food-stuffs differ very greatly in their physical properties. They are, nevertheless, very closely related chemically, since they can all be resolved into the same digestion products. These have been studied with marked success as to their behavior with the digestive juices produced by the pancreas or alimentary tract and with chemical reagents in the laboratory. Certain substances were found to be special products elaborated only by certain tissues and under definite conditions. These became regarded from the standpoint of nutrition as of subordinate interest. Examples of these are the alkaloids, quinin, strychnin and cellulose. This last one serves as skeletal tissue for the plant but is not essential for the animal. In the same category belong the waste products of the life processes of the animal body, such as urea, uric acid and creatinin, most of which are not found in plant tissues.

As early as 1840 proteins, fats, carbohydrates, mineral matter and water were recognized as being never failing components of plant tissues and were looked upon as the fundamental factors in the diet of animals.

Study of Food-stuffs by Chemical Methods.—Investigators in nutrition long labored to discover and to perfect methods for the analysis of food-stuffs, in order to determine the amounts of protein, carbohydrate, fat and ash contained in them. There were observed pronounced differences in the composition of the

many substances which serve as food for man and animal. Meats, eggs and a few seeds, such as the pea and bean, are very rich in protein. The cereal grains contain much less and the fruits, tubers and vegetables contain very little in the fresh condition. Equally great variations are observable in the water content of foods, and in their yields of fats and carbohydrates. One of the great epochs in the development of the science of nutrition is that in which Voit (1881) in Germany, and Atwater and his associates (1895) in America, examined and tabulated in classified form the chemical composition of an extensive list of human foods. Shortly after this, Agricultural Experiment Stations accumulated similar data concerning substances used for feeding animals. Previous to 1900 the idea that there was any marked variation in the quality of proteins from different sources did not become generally appreciated.

Early Evidences of Deficiency Diseases.—It seems remarkable, in the light of the discoveries during the last few years, that close students of nutrition accepted without experimental proof for so long the belief that the results of a chemical analysis revealed the dietary values of food-stuffs.

Primitive man was sufficiently industrious in most parts of the world to secure for himself a varied diet derived from the cereal grains, legume seeds, fruits, roots, tubers, meats and certain edible leaves. From the dawn of the era of his advance toward achievement, he has been in many parts of the world the possessor and protector of flocks and herds. These provided him with clothing and a supply of meat and milk. The importance of this last item in his food supply we have only recently come to appreciate. The famous chemist, Van Helmont, called milk "brute's food," and thought it advisable to substitute for it bread boiled in beer and honey. Baron Liebig likewise proposed in his book "Food for Infants" preparing a substitute for milk.

For centuries in several parts of the world restricted diets of a monotonous character have produced diseases in man. It is of interest now to look back from our present position and point out the problems waiting to be solved.

There had been in the literature of pathology for many years abundant evidences from human experience that a diet may have an appropriate chemical composition and yet be unsatisfactory for the nutrition of man. These observations did not secure from students of nutrition the attention they merited.

It had been known for centuries that certain diets of stale foods produced outbreaks of scurvy among sailors, soldiers and prisoners. It was also known that fresh vegetables and fruits would promptly cure scurvy. No one had made, even in the first decade of the twentieth century, an effort on a comprehensive plan to discover what it was in certain foods which caused them to be incomplete and to induce disease, and what it was in certain foods which made them of specific therapeutic value.

Beri-beri, a peripheral neuritis resulting in paralysis, had been known from remote times. In 1884 it was demonstrated beyond doubt that in the Japanese Navy it was due to faulty diet. Under the direction of Admiral Takaki a ship made a cruise of eight months, during which time the incidence of beri-beri among the sailors was carefully noted. Another ship was then manned and sent on a cruise over the same course with an improved dietary furnished the men. The effects were so striking that the diet of the Army and Navy was improved, with the result that thereafter the disease practically disappeared. In 1897 Eijkman, a Dutch physician, sought to test the question whether there was any relation between the consumption of polished rice and the incidence of beri-beri. He found that pigeons or chickens which were restricted to a diet of milled rice developed in 2 to 4 weeks a condition of paralysis strikingly suggestive of beri-beri in man. He established the relation between milled rice and the disease, and believed that there was something in the bran layer which protected against it. Although several pathologists had repeated and confirmed this observation, it did not influence students of nutrition until Funk called attention to this work in 1910, when he designated the term "vitamine" to indicate a group of indispensable nutritive complexes.

For centuries pellagra has been a scourge among the peasants in some parts of Italy and of France. Its etiology was thought to be due to the poor quality of the simple and monotonous diet. This disease was not observed in America until after 1900. After that time it increased rapidly up to 1917, especially in the southern states.

Human experience had been, however, practically as definite as animal experimentation in demonstrating that scurvy could be cured with certain fresh foods, but not by the same food in the stale condition. As will be pointed out later, there was much difference of opinion as to the cause of scurvy. The vitamins made a great impression on the public mind and justly so.

There is nothing more spectacular than the restoration of a pigeon which has been brought to a condition of helplessness by a diet of polished rice. When a bird is within a few hours of death it can be brought back to an apparently normal condition by the administration of a minute amount of properly prepared substance.

To Funk is due the credit of bringing to the attention the view that there are certain diseases which result from faulty diet, the fault resulting in a lack, relative or absolute, of one or another of several substances, each of which when present in the diet protects against a specific type of pathological change. Beri-beri, scurvy, pellagra and rickets were all attributed by him to lack of "vitamines" in the diet. Actually he had experimental evidence of the existence of only one of these specific "deficiency" diseases, viz., beri-beri, or as it is called when produced experimentally in animals—polyneuritis. This was the only one of the syndromes enumerated which had been studied experimentally in a manner that could be regarded as affording a basis for judgment as to its etiology.

Funk had no clear conception of the importance of the several factors which go to make up an adequate diet. He knew how to produce but one deficiency disease, polyneuritis or beri-beri. This was effected by the method which Eijkman had used in 1897, *i.e.*, restricting pigeons to polished rice as their sole food supply. These observations in pathology failed at that time to impress the students of animal nutrition. Rice is now known to be faulty with respect to at least four dietary factors for the rat, and with respect to five factors for man.

The Wisconsin Experiment with Cattle.—In 1906 an experiment was begun at the Wisconsin Experiment Station which had a far-reaching effect on the minds of students of nutrition. It was planned by S. M. Babcock, and was carried out by Hart and Humphrey (1911), with the later co-operation of Steenbock and McCollum. In this experiment the object was to determine whether rations for cattle so made up as to be alike insofar as could be determined by chemical analysis, but derived each from a single plant, would prove to be of equal nutritive value for growth and the maintenance of vigor.

The ration employed for one group of animals was derived solely from the wheat plant, and consisted of wheat straw, wheat gluten and the entire wheat grain. The ration of the second group consisted of the entire corn plant, which included the

kernel, stalk, and the leaf, together with a portion of corn gluten, a by-product of the cornstarch industry. The third group obtained their ration solely from the oat plant, being fed entirely on rolled oats and oat straw and leaf. There was a fourth group, which it was supposed would serve as controls, which was fed a ration having the same chemical composition, but derived from about equal portions of wheat, corn and oat products.

The animals employed were young heifer calves weighing about 350 pounds, and were as comparable in size and vigor as could be secured. They were restricted absolutely to the experimental ration. They were given all the salt (NaCl) they cared to eat. They were well cared for, and were allowed to exercise in an open lot free from vegetation. Their behavior during growth and during the performance of the functions of reproduction were extremely interesting. All groups ate practically the same amounts of feed, and digestion tests showed that there were no differences in the digestibility of the three rations.

It was not until the animals had been confined to the experimental rations for a year or more that differentiation in their appearance was easily observable. The corn-fed group was sleek and fine, and evidently in an excellent state of nutrition. In marked contrast stood the wheat-fed group. These animals were rough coated and gaunt in appearance. They were small of girth as compared with those fed the corn-plant ration. The weight of the two groups did not differ in a significant degree. The groups fed the oat-plant ration and the mixture of the three plants, leaf and seed, stood intermediate between the lots just described. The assumption that the animals receiving the mixture of products would fare better than the others, and thus serve as the standard group for controls, did not prove correct. The corn-fed animals were at all times in a better state of nutrition than were those receiving the greater variety of food materials.

The reproduction records of these animals are of special interest. The corn-fed heifers invariably carried their young to full term. The young showed remarkable vigor, and were normal in size and able to stand and suck within an hour after birth, which is the rule with vigorous calves. All lived and developed in a normal manner. The young of the wheat-fed mothers were the reverse in all respects. They were born 3 to 4 weeks too soon, and were small, weighing on an average 46 pounds, whereas

FIG. 1.—Photograph of a cow which grew up on a ration derived solely from the corn plant. The seed, straw and leaf of the plant were all included in the food mixture. Her nutrition was excellent, as shown by her appearance, the vigor of her offspring and her ability to produce an abundance of milk. Figure 2 shows a photograph of her calf, taken soon after it was born. Rations consisting of the entire plant may be highly satisfactory. The seed of the plant is never in itself a complete food.

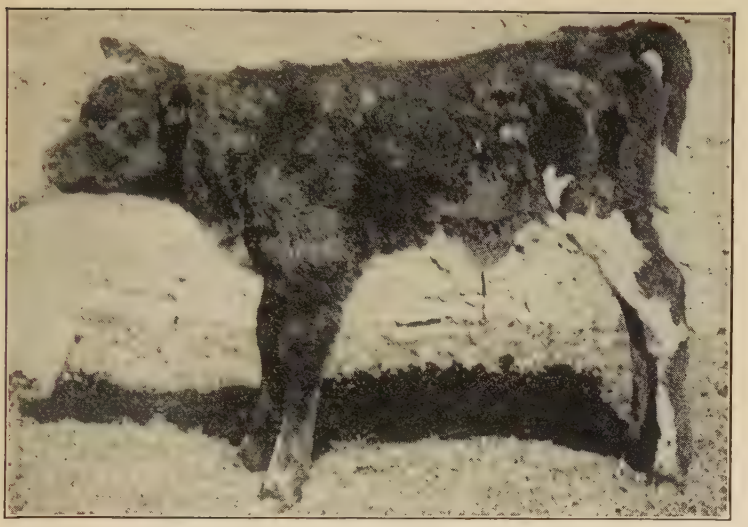

FIG. 2.—Photograph of calf produced by a mother whose ration had been long derived from the corn plant as the sole source of nutriment. It was vigorous and developed normally. The entire wheat plant, seed, leaf and stem make a diet which can support growth, but not good nutrition. The corn kernel alone does not induce growth.

FIG. 3.—This cow was the same age as that shown in Figure 1. She derived her ration entirely from the wheat plant during the last two-thirds of her growing period. This ration, when submitted to chemical analysis, showed almost exactly the same composition as that fed to the cow in Figure 1. Note the poor nutritive condition of the wheat-fed cow. It has not been possible to prepare a ration solely from wheat products, which will induce good nutrition in animals. Figure 4 shows the typical appearance of the calves produced by cows fed upon a ration properly "balanced," but derived entirely from the wheat plant, and containing the seed, stem and leaf.

FIG. 4.—Calf produced by cow shown in Figure 3. It was born prematurely, weighed but little more than half as much as calves normally do at birth, and was dead when born. The importance of the source of the food supply, both for the nutrition of mother and the unborn young, is strikingly illustrated by these animals. (Figures 1 to 4 are from Research Bulletin 17 of the Wisconsin Experiment Station.)

the young of the corn-fed animals weighed 73-75 pounds each. The latter weight is normal for new-born calves. The young of the wheat-fed mothers were either dead when born or died within a few hours. The young of the mothers which had grown up on the oat-plant ration were almost as large as those from the corn-fed mothers, the average weight being 71 pounds. All of the cows in this group produced their young about 2 weeks too soon. One of the four was born dead, two were very weak and died within a day or two after birth. The fourth was weak but with care it was kept alive. The young of the cows which were fed the mixture of the three-plant products were weak in most cases. One was born dead and one lived but 6 days. The mothers were continued on the experimental rations and the following year they repeated in all essential details the reproduction records observed in the first gestation period.

Records were kept of the milk production during the first 30 days of the first lactation period. The average production per day per each individual of the corn-fed lot was 24.03 pounds; for the wheat-fed animals 8.04 and for the oat-fed animals 19.38 pounds. Those fed the mixture of the three plants produced an average of 19.82 pounds of milk per cow per day during the first 30 days. In the second lactation period the average figures for milk production were 28.0, 16.1, 30.1, and 21.3 pounds, respectively, per day during the first 30 days.

Through autopsy and analysis of the tissues of the young, and analysis of the feeds and excreta of the animals of the several groups, an elaborate attempt was made to solve the problem of the cause of the marked differentation of the animals fed these restricted diets. Interesting data were secured which showed marked differences in the character of the fat in the milk of the cows of the different lots. The urine of the wheat-fed animals was invariably distinctly acid in reaction, whereas that of the other lots was alkaline or neutral. It was not possible by any means known to biological chemistry to discover the cause of the pronounced differences in the physiological well-being of the different lots of cows.

The Problem of the Purified Food Mixture.—This experiment confirmed McCollum's conviction that the only way in which the problems of nutrition could ever be solved was to solve the problem of the successful feeding of the simplified diet. He believed if this was accomplished it would be possible to proceed from the simplest diet to the complex diets employed

in practical nutrition. It would also be possible to ascertain the nature of the dietary faults in each of the natural foods, the seed alone and the leaf alone, before attempting to interpret the cause of malnutrition in animals fed the more complex mixtures.

Several investigators had been unsuccessful in their efforts to nourish young laboratory animals on mixtures of purified protein, starch, sugar, fats and mineral salts. Lunin (1888) was the first to investigate the significance of inorganic salts in animal nutrition. In his experiments he found that adult mice could live for several months in good health on a diet of milk, but they invariably died within a month if they received a ration composed of what he believed to be the essential ingredients of milk, *i.e.,* casein, butter fat, milk sugar and the ash of milk. Commenting upon his experiments he says, "Mice can live quite well under these conditions when receiving suitable foods (*e.g.,* milk), but as the above experiments demonstrate they are unable to live on proteins, fats, carbohydrates, salts and water, it follows that other substances indispensable for nutrition must be present in milk besides caseinogen, fat, lactose and salts." Although Bunge gave this conclusion publicity in his *Physiological and Pathological Chemistry,* a text book widely used at one time, it did not attract the attention it deserved. Others who attacked the problem of the significance of the mineral content of the diet were Steinitz (1898), Leipziger (1900), Zadik (1899), Ehrlich (1900), Socin (1891), Hall (1896), Falta and Nöggerath (1905), Jacob (1906), Marcuse (1896), Henrique and Hansen (1905), and Willcock and Hopkins (1906). In all these trials directed toward the nutrition of animals with food mixtures which contained everything determined in the ordinary food analysis, the animals lost weight steadily from the beginning of the experiments. They died unless their diet was changed. It was impossible at the time to explain the reason for their failure.

Studies of this character must of necessity be carried out with small laboratory animals, because it is difficult and laborious to prepare the purified food-stuffs in sufficient amounts for the conduct of feeding experiments. It is highly desirable to shorten the length of such experiments as far as possible by the employment of short-lived omnivorous animals which grow rapidly and which quickly reach maturity. In this way it is possible to accumulate data sufficiently fast to make progress reasonably satisfactory. The domestic rat serves this purpose admirably. It

has a gestation period of 21 days and under normal conditions the young are ready to wean at the age of 25 days. A well-nourished female usually produces her first litter at about the age of 90 days; she will have five or more litters by the time she reaches an age of 14 months, which age ordinarily marks the end of her period of fertility. The extreme span of life of the well-fed rat is about 36 months. By using such an animal it is possible within a relatively short time to accumulate data regarding growth and reproduction which it would take years to secure with domestic animals of large size, long period of gestation and long span of life. With the latter the expense would be prohibitive. In 1907 McCollum took up the study of the cause of the failure of animals to grow on mixtures of purified food-stuffs and employed the domestic rat as the experimental animal.

Can Inorganic Phosphorus Compounds Meet all Physiological Needs?—The titles of several of the papers cited in connection with the feeding of isolated and purified food substances show that one of the earliest explanations occurring to investigators as the possible cause for the failures was the probable need of the animal for phosphorus in certain organic combinations. Some of the experiments were interpreted as supporting the view that proteins containing phosphorus were better utilized for growth than were phosphorus-free proteins. Between 1895 and 1910 there was a wide-spread belief among students of nutrition that the presence of organically bound phosphorus might be a factor of great importance. It is easy to appreciate how the idea gained credence. Casein, the principal protein of milk, is a phosphorized protein, and the yolk of egg is very rich in the phosphorized protein, vitellin, and in phosphorized fats. Milk and the egg yolk are peculiarly constituted for the rôle they play in reproduction and the nutrition of the young during the earliest period of post-natal life when growth is most rapid. It seems significant that in these two substances organically bound phosphorus is very abundant, whereas it is not abundant anywhere else among our natural foods. It has never been shown conclusively up to the present time that a protein occurs in plant tissue which contains the element phosphorus as a part of its molecule. Other organic phosphorus compounds (lecithins, nucleic acids) do exist in plant tissues but never in great abundance.

Among the experiments cited in which animals were confined

to diets of proteins, carbohydrates, fats and mineral salts, those of Hall, Steinitz, Leipziger, Zadik and Ehrlich were directed toward ascertaining the importance of phosphorus in organic as contrasted with inorganic combinations. Other investigators working along the same lines were Kornauth (1900), Gottstein (1901), Ehrstrom (1903), Hirschler and Terray (1905), Gilbert and Posternak (1903), Tunnicliffe (1906), LeClerc and Cook (1906), Koch (1906), Hart, McCollum and Fuller (1909) and Forbes (1914). The data seemed definitely to support the view that organic phosphorus compounds were essential in the diet or else they failed to establish that they were dispensable.

Mistaken Conclusion which Stimulated Research in New Direction.—McCollum (1909) introduced a new feature into experiments of this type by seeking to increase the variety of food-stuffs in the diet as far as possible. He hoped in this way to overcome the failure of others to secure satisfactory nutrition in animals. In the series of experiments referred to the one condition which was required of every organic component of the ration was that it should be free from phosphorus in any form. This condition was practically met. The only source of phosphorus in the diet was finely ground tricalcium phosphate a natural mineral.

The proteins employed in these experiments were edestin from the hemp seed and zein from maize. Cornstarch, wheat starch, milk sugar, glucose, cane sugar, butter fat, bacon fat, and cholesterol, together with the ash constituents, made up the food supply. Cholesterol is a substance found in most body tissues and has physical properties similar to fats. No effort was made to feed a constant mixture. On the contrary, the diet was changed from day to day as far as possible in order to prevent the dreaded loss of appetite. Several young rats gained in weight, one somewhat more than doubling its weight in 56 days. The rate of growth would now be considered far below normal. These results were interpreted to mean that growth had actually been induced by a mixture of purified food substances, where all previous attempts had failed. The only difference between the experimental diet used in this study and those of the earlier investigators appeared to be in the palatability. Important deductions were drawn from these results concerning certain synthetic reactions which the body tissues could accomplish.

The above paper is deserving of special notice because it reported the first experiments involving growth with a food

supply which was at that time considered to be essentially a mixture of purified food-stuffs, every one of which could be named. It seemed to demonstrate that the view which had long been held that the only substances necessary in the diet are proteins, carbohydrates, fats and certain salts was well founded. It appeared to prove that the cause of the failure of the earlier experiments of this type was due to lack of palatability of the food, which led to failure of the animals to ingest sufficient amounts. This was one of the conclusions of the author, but it has since become apparent that this was an error. The data disposed of the disputed question concerning the relative merits of phosphorized as compared with phosphorus-free proteins. They could be interpreted only to mean that the animal body is capable of synthesizing from salts of inorganic phosphoric acid all of the complex phosphorus-containing organic substances which are so important as components of living matter.

These ideas being accepted as proven facts it seemed that the next great advance in the field of nutrition was in the study of the relative value of the numerous proteins of the plant and animal tissues. Organic chemists showed them to be very differently constituted when derived from different sources. The chemical methods were not entirely satisfactory for the analysis of these complex bodies. Even in the hands of the most skilled chemists, among whom Kossel, Fischer, and Abderhalden in Germany, and Osborne in America were the most noted, only a little more than half of the protein molecule could be accounted for in the form of identifiable products of digestion, the amino-acids. Obviously such analyses, although representing an advance in chemical science, were too incomplete to permit of using them as a basis of judgment as to the nutritive values of individual proteins. The way now seemed open for the quantitative comparison of isolated and purified proteins. A single protein could be fed as the sole source of the dietary factor.

Unfortunately for the progress of this branch of physiological chemistry the diet with which McCollum had secured growth in young rats was something more than it appeared to be. Five years later it became apparent that the cause of the growth of this group of animals lay in the chance inclusion in the diet of milk sugar, which was not as pure as it was supposed to be, and to the inclusion of butter fat. This diet was not actually a simple mixture of proteins, several carbohydrates, fats and inorganic salts, but contained as impurities two nutritive prin-

ciples which were unsuspected. One of these was in the milk sugar, although this was believed, on the basis of careful tests, to be highly purified lactose; the other was an uncharacterized substance which the butter fat contained. The failure of all investigators in this field to appreciate this led to confusion for several years. The further history of investigations which cleared up this problem will be given in the following chapter

CHAPTER II

A BIOLOGICAL METHOD FOR THE ANALYSIS OF A FOODSTUFF

Osborne and Mendel's Studies on the Purified Food Mixture.—It was pointed out in the preceding chapter that repeated failures characterized experimental studies in which animals were confined to diets containing only the well known food principles. These failures had practically established the belief that this line of study was unprofitable, because all animals, for some unknown reason, died when confined to such a regimen. The moderately successful results of McCollum with such mixtures aroused hope that a new and valuable body of data could be secured which would reveal the sources of the valuable and less valuable proteins among our natural foods.

In 1909 Osborne and Mendel took up this line of investigation and proceeded to feed individual proteins with a diet which was otherwise composed of starch, lard and mineral salts. At the outset of their studies they gave credence to the evidence which McCollum had presented that such diets could be successfully fed to animals. They planned to interpret on the basis of the capacity of young rats to grow on a constant diet, varied in successive experiments only with respect to its protein, the relative merits of such proteins as tissue builders. They did not, however, actually duplicate the experimental conditions of McCollum, but adopted a diet which they regarded as having essentially the same properties. This consisted of purified protein, starch, lard and a salt mixture. They met with complete failure in these attempts to nourish their animals with such a diet. They tried a salt mixture recommended by Röhmann (1908) with which he had had success with a diet consisting in great measure of purified food substances. They also used the salt mixture described by McCollum. In all cases, however, their animals declined steadily from the time they were confined to such diets. They proceeded a step farther and established the fact that the failure of the animals was not due to lack of

appetite, since the food intake was found to be sufficient to promote growth provided the composition of the food had been satisfactory. At that time no one was able to see any important difference between the quality of a purified protein, three carbohydrates, *one being milk sugar,* two kinds of fats, *one being butter fat,* and a salt mixture, as compared with a simple mixture of protein, starch, lard and salts (McCollum, 1909).

Protein-free Milk.—Since Osborne and Mendel failed to nourish animals on a diet of isolated and purified food-stuffs they adopted what they apparently regarded as essentially the equivalent of a mixture of purified food-stuffs. The basis of their new diet was 28 per cent of "protein-free milk." This product was made by removing as completely as possible the fat, casein and lactalbumin from milk, and evaporating the resulting whey to dryness. The residue was a yellow solid, easily powdered. It consisted for the most part of milk sugar and the mineral salts ordinarily present in the milk. In fact, no other components of the product could be named. "Protein-free milk" was found to contain about 0.7 per cent of nitrogen, but the form in which it was present could not be ascertained.

They prepared diets consisting of 28 per cent of this product, the other components being starch, lard, agar-agar and the protein which they desired to study. This combination had certain properties which could not be accurately defined, but which made it far superior to any of the simple mixtures used by others. With these diets they were able *with certain proteins* to secure in rats normal growth to the full adult size. The results, judging from the widespread comments on these experiments, were regarded by many as the successful accomplishment of the quantitative comparison of the nutritive values of purified proteins.

Artificial Protein-free Milk.—Since Osborne and Mendel found their diet containing "protein-free milk" suitable for the purpose which they had in view, viz., the comparison of the relative values of various isolated proteins, they apparently abandoned further efforts to solve the problem of what was lacking in their former diets of purified food-stuffs. They did prepare an "artificial protein-free milk" from what they regarded as pure milk sugar and a mixture of mineral salts made up from reagents. An attempt was made to imitate exactly the composition of "natural protein-free milk." With this mixture in place of the natural product they secured excellent growth records over periods covering two months or a little longer

(1913). They made a special effort to secure lactose and salts of a high degree of purity for certain of their experiments in which "artificial protein-free milk" was used. This preparation gave negative results in most cases, and in all the experiments growth was decidedly less than when materials of ordinary purity were used. They carried their investigations in this direction no further than to make the addition of traces of iodin, manganese, fluorin and aluminum. These impurities, suspected in their reagents of poorer quality, actually improved the diet, making it capable of inducing better and more continuous growth, but the animals remained under-sized. Osborne and Mendel were inclined to accept the view for a time that the peculiar virtue of their natural "protein-free milk" lay in the nice adjustment of the inorganic elements and radicals it contained.

Osborne and Mendel (1911) reported their failure to induce growth in rats on a diet composed of casein, starch, lard and the inorganic salt mixtures employed by Röhmann and by McCollum. It was evident from their records of food intake that sufficient food had been consumed to promote growth if its quality was satisfactory.

Studies of Purified Food Mixtures Confusing.—There was no apparent explanation for their failure. The diet on which McCollum had reported growth consisted of two pure proteins, several carbohydrates, including milk sugar, two fats, including butter fat, and a salt mixture (see p. 18). McCollum had erroneously supposed that the success observed with this food mixture was due to palatability and consequently adequate food consumption. An effort was at once begun by him to discover wherein lay the lack of harmony in these observations. As the situation stood, his work was discredited, and he felt that many would be inclined to regard his reported data as falsified. It was now imperative that he should satisfactorily explain why others had failed to confirm his results.

He found his results irregular and confusing on returning to this field of study. He and Miss Davis, using a diet which consisted of casein, dextrin, milk sugar and salts, tried the addition of different kinds of fats to see if any special quality could be found in any of these. In 1912-13 they found that rats restricted to such a diet grew when butter fat or egg yolk fats were incorporated in the food. Olive oil or lard did not under these conditions induce any growth. These results were de-

scribed in June, 1913. They interpreted their results to mean that there was in certain fats a dietary essential which had not hitherto been recognized.

Synthesis of Complex Lipins by Animal Body.—McCollum and Davis appreciated the fact that the animal body is capable of producing synthetically the complex lipins, or fat-like substances such as lecithin, which in addition to glycerol and fatty acids contains phosphoric acid and a nitrogenous base called cholin. McCollum, Halpin and Drescher (1912) had shown that young hens could grow for a considerable time on a diet free from this class of substances. While confined to it hens produced many eggs in which were contained much more of the complex lipins than the bodies of the hens could furnish. The birds were in good health at the end of the experiment. Since butter fat is free from lecithin and related substances, McCollum and Davis interpreted their data to mean that the stimulating effect of butter fat on growth could not be attributed to any known lipin.

Osborne and Mendel's Fat-free Diet.—Osborne and Mendel (1912) published a paper describing successful nutrition with diets which contained no fats and only traces of substances soluble in fat solvents such as ether. This paper tended greatly to confuse the entire subject. They fed a diet consisting of protein, cane sugar, starch and "protein-free milk." In some of their experiments with similar diets "artificial protein-free milk" was used instead of the natural product. The artificial product was composed of milk sugar and a salt mixture from reagents. It was made in close imitation of the mineral content which analysis showed to be present in the natural product.

In commenting on these results Osborne and Mendel stated: "Employing the methods which were adopted in our earlier feeding experiments with isolated food substances, we have succeeded in inducing a normal rate of growth in white rats with dietaries devoid of fat throughout almost the entire period during which growth ordinarily continues." They stated that "McCollum has demonstrated that the phosphorus needed by an animal for phosphatid formation can be drawn from inorganic phosphates, and that phosphatids can be synthesized anew in the animal body. Röhmann asserts the possibility of lecithin synthesis in mice which were maintained into the second generation on lecithin-free food. Our own experiments point in the same direction with regard to the lipoids in general, and they give positive evidence of the dispensableness of true fats for growth."

It appears that they did not regard their failure with purified diets as being due to the absence of accessory factors. This may be assumed from their statement that they had achieved a considerable degree of success in feeding in the absence of the hypothetical vitamins. Hopkins and Neville (1913) challenged their statement and attempted to repeat their experiments. Using carefully purified ingredients they were unable to obtain any appreciable growth on such diets. The animals, in spite of satisfactory consumption of food, were all dead before the expiration of 40 days. In confirmation of the earlier experiments of Hopkins it was found that when 2 c.c. of milk, per day, was given, in addition to the basal diet, this failure was avoided.

Unique Dietary Property of Butter Fat.— When Osborne and Mendel's paper appeared McCollum and Davis had already accumulated experimental data which led them to believe that there were great differences in the nutritional value of fats from several sources. With diets composed of protein, starch, milk sugar, salts and fats, it had been found that prolonged growth was secured when butter fat was added. This had been dissolved in ether and passed through filter paper in order to make certain that no traces of any ingredients of the milk except fats and substances having the same solubilities were present. Egg yolk fats behaved like the purified butter fat, whereas little growth and early failure resulted when olive oil or lard were the only fats in the diet. Owing to an epidemic which destroyed the rat colony a year elapsed before a demonstration of this fact could be secured. McCollum and Davis (1913) recorded their findings that purified butter fat, and likewise egg yolk fat, contain something which greatly promoted growth, and that lard and olive oil did not possess this property.

Osborne and Mendel's Observations on Butter.—Osborne and Mendel had discovered by this time the error of their conclusion that growth and well-being could be secured in animals restricted to a diet free from fats and containing "but an insignificant trace of ether extract." The month following the appearance of the paper by McCollum and Davis demonstrating the peculiar value of butter fat and egg yolk fats as contrasted with lard and olive oil (1913) Osborne and Mendel published a paper on "The Relation of Growth to the Chemical Constituents of the Diet" (1913). They described in this paper the preliminary period of growth they secured with a diet of purified protein, starch, sugar and "protein-free milk." This short period of

growth, which extended between 60 and 120 days, was followed by decline. They found that the decline could be checked by the addition of 16.4 per cent of butter. Animals which received the butter from the beginning of the experiment grew uninterruptedly to maturity. In their paper on nutrition with fat-free food-stuffs they had, curiously enough, interrupted their experiments at the end of about 140 days from the time when the animals were restricted to the "protein-free milk" and "artificial

CHART I

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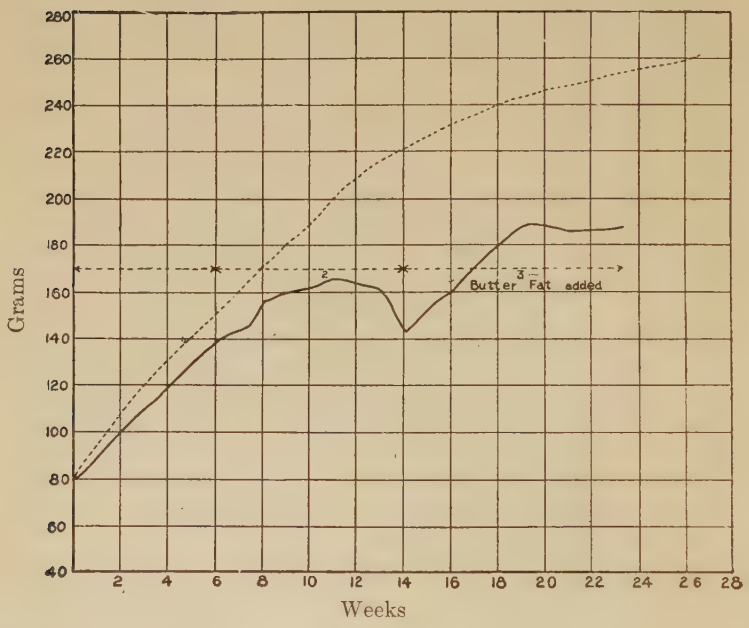


protein-free milk" mixtures. They described these records as successful growth "through almost the entire period."

It was not possible to draw any conclusions from the data presented in their paper now under discussion as to whether the unknown food complex present in butter was in the fat fraction or in the non-fat fraction of the latter. Butter contains about 15 per cent of milk substance other than fat. This portion consists, among other things, of water, protein, cells from the mammary gland, leucocytes and bacteria. It was impossible to decide

without further evidence whether it was in some of these or in the fat itself that the substance occurred which exerted such remarkable effects on the growth of animals. Hopkins (1912) had reported that rats restricted to diets of purified food-stuffs steadily declined, whereas similar ones which were confined to the same diets, but in addition received 1 to 3 c.c. per day of fresh milk, were able to grow. Even after decline had set in on the basal diet it could be checked by this small quantity of milk, which in no instance exceeded 4 per cent of the dry weight of the diet.

CHART I (*Rat* 141, *male*) shows the record of a rat which grew continuously although slightly under normal rate during 80 days on a ration of relatively pure food substances. There was at this time a rapid decline in body weight. The addition of 10 per cent of ether-soluble butter-fat to the diet led to a prompt resumption of growth during the following 35 days, when the rat gained 50 grams. The rations employed were as follows:

Period I		Period II		Period III
	per cent		*per cent*	
Salt mixture	6	Salt mixture	8	Same as II with butter fat replacing part of dextrin.
Casein	12	Casein	12	
Lard	20	Lactose	20	
Lactose	15	Dextrin	61	
Starch	42	Agar-agar	2	
Agar-agar	5			

The salt mixture employed consisted of:

	grams		*grams*
Sodium chlorid	0.61	Calcium lactate	11.38
Dipotassium phosphate	17.00	Magnesium citrate (10.2% Mg)	23.42
Monocalcium phosphate	1.63	Ferric citrate	1.00

The lactose in the diet was not entirely pure, and contained enough of the vitamin B to support growth.

The curve in Chart I represents the original growth record published by McCollum and Davis, which led them to the discovery of the special growth-promoting properties of butter fat as contrasted with vegetable fats and certain animal fats.

Osborne and Mendel's Studies on Butter Fat and Cod Liver Oil.—Five months later Osborne and Mendel (1913) described experiments entirely analogous to those of McCollum and Davis. They fed their animals protein, starch, "protein-free milk," lard and *butter fat.*

Although the final evidence which Osborne and Mendel offered

as proof of the unsuspected value of butter fat in nutrition was of the same kind as had been presented 5 months earlier by McCollum and Davis, in which *butter fat* and *egg yolk fats* were shown to be similar, and superior to lard or olive oil, their paper appeared to misinterpret the work of McCollum and Davis. They stated that "In none of their (McCollum and Davis) published records was the recovery so rapid as in most of ours, nor was the rate or extent of growth, after reaching the previous maximum weight, any greater than on the butter fat-free diet earlier supplied." Further, "Although the data furnished by McCollum and Davis strongly indicate that butter fat has a marked influence on growth, they by no means prove that butter fat contains something essential for the metabolism of growth apart from that of maintenance."

It is now clear that the reason for the better growth of Osborne and Mendel's animals when butter fat was added to the diet was due to the fact that their "protein-free milk" contained an abundance of a second dietary factor, the anti-neuritic substance which prevents beri-beri, whereas the diets of McCollum and Davis contained decidedly less of this substance which was present as an impurity in the supposedly pure milk sugar which their diet contained. It seems certain that there is no substance essential for maintenance, apart from growth, as the last quoted sentence suggests. This idea has been in the minds of several investigators, but the evidence that normal maintenance can be secured on any diet lacking in a complex necessary for growth is very slender indeed. This point will be discussed in Chapter IV.

Osborne and Mendel (1914) confirmed the observation of McCollum and Davis that egg yolk fats had the same effect on growth that butter fat had, and that lard did not stimulate growth as did egg and milk fats. They investigated almond oil and found it to resemble olive oil in the respect that it did not promote growth. They further demonstrated that cod liver oil contained the dietary principle found in butter fat and egg yolk fats.

Indispensability of Certain Lipins.—The results of these investigations recalled the fact that as early as 1909 Stepp had described experiments in which he had found that bread prepared with milk was capable of maintaining adult white mice without loss of weight for an indefinite period. When the same bread was extracted with alcohol-ether, the animals restricted

to it rapidly declined. Replacement of the extracted material failed to restore the dietary properties of the original bread in those cases where the extracts had been heated for several days. When care was exercised to avoid high temperature or prolonged heating, the addition of the extracts made the extracted bread a complete food. In later experiments Stepp showed that the addition of the salts which were extracted with the lipoids did not prevent the rapid decline of the animals. *Butter additions did not prevent decline,* but the addition to the extracted bread of lipin extracts of egg yolk, bran, dry milk or wheat, restored the extracted bread to dietary completeness. This led Stepp to suggest the existence of an unidentified indispensable dietary unit, which he appears to have regarded as a member of the lipoid class. He was unable to identify this substance with cholesterol, lecithin, kephalin and cerebron. All members of that class of substances failed to restore the nutritive value of the extracted food-stuffs. He states "It is not impossible that unknown substances indispensable for life go into solution with the lipoids, and that the latter thereby become what may be termed carriers for these substances."

Confusion Concerning the Essential Nutritive Factors Previous to 1915.—It has been pointed out that students of normal nutrition failed to make use of the observations of the pathologists during the decade between 1905 and 1915. In discussing the possible explanation of the early failure of their animals fed isolated food-substances, together with "artificial protein-free milk," Osborne and Mendel (1913) emphasized the improvement of the latter by the addition of traces of manganese, iodin, fluorin and aluminum, and dwelt upon the possible importance of minor variations in the inorganic moiety of the diet. Even at the end of 1913 in commenting upon McCollum's studies of butter fat they stated "The added butter fat may have simply supplied something analogous to the so-called vitamines, which Funk considers to be essential for life, and thereby enabled the animals to resume growth on a food thus made adequate for maintenance." Further, "It is still rather early to generalize on the rôle of accessory 'vitamines' when the ideal conditions in respect to the familiar fundamental nutrients and inorganic salts adequate for prolonged maintenance are not completely solved." These quotations will serve to show that these investigators were likewise in the same state of confusion as to the interpretation of the data in the literature of pathology which described the

experimental production of beri-beri and of scurvy as were McCollum and his co-workers.

Experiments of Hopkins with Purified Food Substances.— As early as 1906 Hopkins wrote "But further, no animal can live upon a mixture of pure protein, fat and carbohydrate, and even when the necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or other animals, and these contain countless substances other than the proteins, carbohydrates and fats. Physiological evolution, I believe, has made some of these well nigh as essential as are the basal constituents of diet; lecithin, for instance, has been repeatedly shown to have a marked influence upon nutrition, and this just happens to be something already familiar, and a substance that happens to have been tried. The field is almost unexplored, only it is certain that there are many minor factors in all diets, of which the body takes account. In diseases, such as rickets, and particularly in scurvy, we have had for long years knowledge of a dietetic factor, but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure. They are, however, certainly of the kind which comprises these minimal qualitative factors that I am considering. Scurvy and rickets are conditions so severe that they force themselves upon our attention, but many other nutritive errors affect the health of individuals to a degree most important to themselves, and some of them depend upon unsuspected dietetic factors."

In 1912 Hopkins published the results of experiments extending over several years. Rats fed upon a food mixture containing caseinogen, starch, cane sugar, lard and inorganic salts remained alive and grew a little when the components of the diet were supplied in a crude condition. When they were carefully purified growth invariably ceased after a short period, and the animals declined and died. Hopkins showed that the energy consumption of the rats was sufficient so that their failure was not due to insufficient food intake. It was found that growth ceased at a time when the animals were consuming food in more than sufficient quantity to maintain normal growth.

The addition of a small daily allowance of milk, which in no case supplied more than 4 per cent of the food eaten, induced normal growth. These experiments marked the beginning of an appreciation of the importance of what Hopkins has termed the

accessory factors of the diet. It appears that at this time Hopkins was not aware of the epoch-making observations of Eijkman that a diet of polished rice would induce polyneuritis in birds and that rice polish contained something which would relieve the condition. (1897.)

Formulation of Biological Method for Analysis of a Foodstuff.—It is easy now to see in the light of later experience why such skepticism existed regarding the necessity of hitherto unappreciated factors in the diet. Osborne and Mendel, and McCollum, had seen young rats grow for a considerable time when restricted to diets of supposedly pure food substances. These substances, however, always contained a considerable amount of milk sugar, and it was not appreciated at the time that impurities of a most important nature might still adhere to lactose of a relatively high degree of purity.

When it was found in 1913 that the addition of butter fat promoted growth on a diet which consisted, aside from this fat, only of substances of accepted purity, every component of the diet being regarded as known chemically, it seemed very plausible that the only unidentified dietary essential for the rat was associated with the fat fraction. This view was at one time held by McCollum. In all such studies the question as to the degree of purity of each of the ingredients of the diet necessary to warrant the conclusion that no impurities of a significant nature were present could be decided only through critical and comparative experiments.

The first significant advance in solving the problem concerning the number of unidentified nutrient principles essential in the diet of the rat is of historical interest. During the years 1913 to 1918 Osborne and Mendel, and McCollum and his coworkers confined their researches to different fields. The former, believing that their diet containing a protein and other isolated foodstuffs, supplemented with 28 per cent of "protein-free milk," was satisfactory for the study of the comparative values of the proteins from various sources, extended their investigations in the direction of determining the biological value of each of the more important proteins isolated from natural foods. McCollum and his coworkers turned their attention to the determination of the nature from the dietary standpoint of the deficiencies of our natural foods. It was through the latter studies that the next advance was gained in our knowledge of what constitutes a satisfactory diet.

While the studies with rations restricted to a single plant source were in progress with cattle at the Wisconsin Experiment Station, McCollum, beginning in the year 1907, restricted the diet of rats to each of the more important single grains as the sole source of nutriment. It was discovered that whole wheat, rolled oats, maize, or any other seed fed singly, failed to induce any growth in young animals, or to maintain life for a long period of time. It seemed in 1913 after the completion of the experimental studies described that the necessary knowledge was available to enable one to determine the nature of the faults responsible for these failures.

Chemical Analysis versus Biological Analysis of Food-stuffs.—Chemical analysis showed the cereal grains to contain all the essential food substances for which we know how to analyze. *It was assumed as a working hypothesis that the only unknown factor contained in the diet was that associated with certain fats.* The first intensive study was made on the wheat kernel. It was reasoned that since all the types of nutrients are represented in a seed such as wheat, except possibly the unknown one which had been demonstrated to be present in certain fats, the fault or faults of wheat from the dietary standpoint must lie in the *quality* of one or more of these food factors. It was believed that valuable data might be secured by enhancing the protein, the inorganic moiety and the fat factors separately in feeding experiments. It was thought that by a systematic series of feeding experiments one would be able to discover which was the limiting factor for growth in this seed. This possibility was investigated by enhancing the quality of one nutrient principle at a time. Accordingly, McCollum and Davis fed the wheat kernel in the following combinations, and obtained the results noted. This procedure has since been known as the biological method for the analysis of food-stuffs.

1. Wheat alone....No growth, short life.
2. Wheat plus purified protein....No growth, short life.
3. Wheat plus a salt mixture which gave it a mineral content similar to that of milk....Very little growth.
4. Wheat plus a growth promoting fat (butter fat)....No growth.

From these results it seemed apparent that either the working hypothesis concerning the factors which are necessary in the diet must be wrong, or there must be more than a single dietary factor deficient in wheat, and jointly responsible for the poor nutrition of the animals. In order to test this theory another series of

experiments was carried out, in which wheat was supplemented with two purified food additions:

5. Wheat plus protein, plus the salt mixture....Good growth for a time. Few or no young. Short life.
6. Wheat plus protein, plus a growth-promoting fat (butter fat)....No growth. Short life.
7. Wheat plus the salt mixture, plus a growth-promoting fat (butter fat)....Fair growth for a time. Few young. Short life.

The behavior of the animals fed wheat with two purified food additions suggested that there were three dietary factors of poor quality in the wheat kernel. This was demonstrated to be true by a feeding trial in which wheat was fed, supplemented with three purified food additions:

8. Wheat plus protein, plus the salt mixture, plus a growth-promoting fat (butter fat)....Good growth, normal number of young, good success in rearing young. Life approximately the normal span. See Chart II.

These experiments were conducted in 1914. They seemed convincing to McCollum and Davis that the only element of mystery in the normal diet was the unidentified substance in butter fat. With the improvement of three dietary factors wheat became a satisfactory food for the nutrition of an animal during growth, and for the support of all the functions of reproduction and rearing of young.

This series of experiments brought to light two new viewpoints in nutrition. One of these was—*the inorganic content of the wheat kernel, although it furnishes all the necessary elements, does not contain enough of certain of these to meet the requirements of a young animal during the growing period.* It is true that some years earlier Henry (1889) had called attention to the deficiency of the corn kernel in ash content and had added wood ashes to the diet in some of his experiments. He noted improvement in the well-being of the animals. The fact that a seed, such as wheat, failed to supply sufficient inorganic elements was not generally appreciated and was given but little attention in books on nutrition. Later work by McCollum and Simmonds demonstrated that the deficiency in mineral elements in wheat and other seeds is limited to four elements, calcium, phosphorus, sodium and chlorin (1917, 1921).

A second new viewpoint which was brought out by these experiments was—the wheat kernel is too poor in its content of the unidentified substance which butter fat contains to nourish satisfactorily an animal over a long period of time.

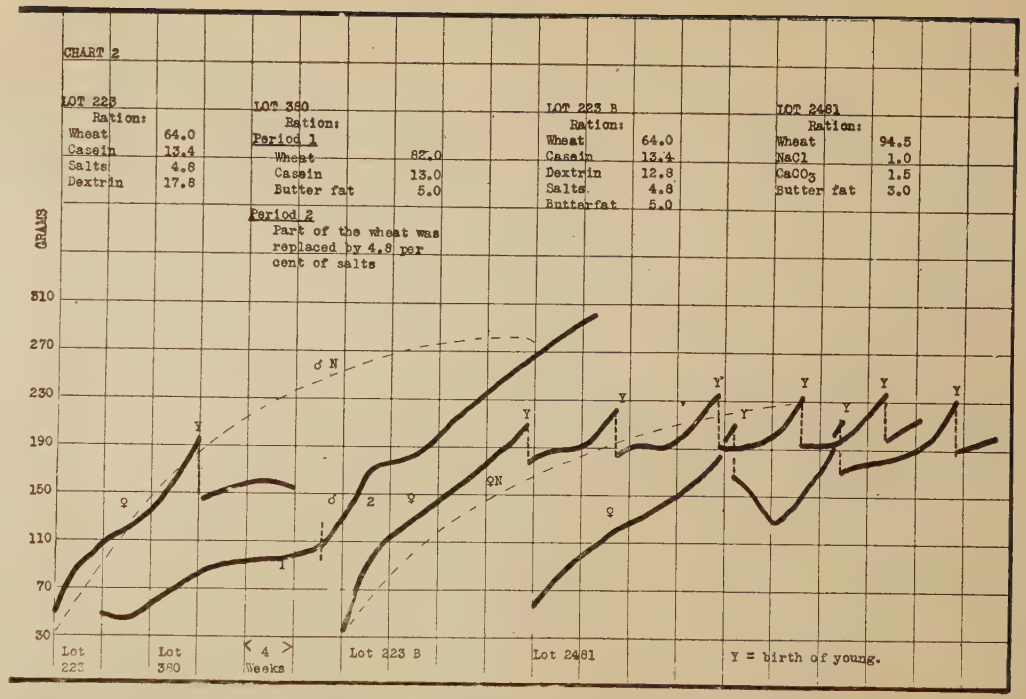


CHART II.—This series of curves illustrates the nature of the deficiencies of the wheat kernel from the dietary standpoint. Lot 223 lacked sufficient vitamin A but was otherwise complete. Lot 380 contained everything which was essential except certain mineral salts (Period 1). In Period 2 the animals became able to grow because of the inclusion of the missing calcium, sodium and chlorin. Lot 223 B grew well and were very fertile and successful in rearing young on wheat supplemented with respect to protein, mineral salts and vitamin A (in butter fat). The record of the animal on ration 2481 shows clearly that the mineral deficiencies of wheat are most serious, for without enhancing the proteins of this cereal, good growth and moderate fertility were secured. This curve further shows that only a calcium salt and common salt or sodium chlorid are necessary to complete the inorganic moiety so as to make possible fairly satisfactory nutrition.

Deficiencies of Polished Rice.—The above investigations were carried out in 1913, soon after the publication by Funk of his first work on polyneuritis. In the previous year Hopkins had called attention to the remarkable effects produced by the addition of small amounts of milk to diets composed of purified food-stuffs. The vitamin hypothesis had just been formulated by Funk (1913). McCollum and Davis were aware, therefore, of the relation of a diet of polished rice to experimental beri-beri. They believed, in the light of their experiences with the diet of purified protein, milk sugar, fats and inorganic salts, that such a mixture was capable of inducing growth when certain fats were supplied, but not when others were substituted. The further fact that wheat could be supplemented by purified protein, a growth-promoting fat, and a suitable salt mixture, that is, with food-stuffs of known character, seemed to indicate that there was but a single unidentified substance necessary in the diet. They decided to apply to polished rice the same procedure which had shown so clearly the nature of the dietary deficiencies of wheat. They reasoned that rice could be nothing less than a mixture of proteins, starch, fat and inorganic salts, similar to that contained in wheat, but in different proportions. It seemed to them that polished rice could be supplemented with a suitable salt mixture, a purified protein and a growth-promoting fat, so as to induce growth and maintain animals for a long time in a state of health. This seemed to be a necessary corollary, since they had secured growth and well-being in animals fed solely upon a mixture of purified protein (casein), starch, milk sugar, butter fat and a mixture of inorganic salts of suitable composition (1913).

It was a great surprise to McCollum and Davis to find that polished rice supplemented with the protein, casein, butter fat and a properly constituted salt mixture, failed to induce growth in young rats (1915). Not only did they fail to grow, but in the course of a few weeks they developed in some cases a state of paralysis which was suggestive of polyneuritis. Here was an apparent contradiction. The polished rice could be nothing less than a mixture of protein, carbohydrate, fat and salts. The only difference between this and the mixture of supposedly purified food-stuffs with which they had observed growth was in the 20 per cent of milk sugar which the synthetic diet contained.

They, therefore, decided to repeat the experiments with the latter mixture, with the milk sugar replaced by starch. It was

found that this change in the composition of the food mixture made the difference between success and failure. No growth could be secured when the milk sugar was omitted. Later experiments showed that if milk sugar sufficiently purified by repeated crystallization was added to the purified food mixture it was no longer effective in inducing growth, whereas the water from which the sugar had been crystallized would, when evaporated upon the food mixture, render it capable of inducing growth. This made it evident that there is a second dietary essential, of which an animal needs but a very small amount, but which is absolutely necessary for growth in the young and for maintenance of health in the adult.

Further experiments were then conducted to find whether this unidentified substance which was being added accidentally as an impurity in the milk sugar was the same as the one which Funk had discovered in his studies of beri-beri. It was found that pigeons which had developed beri-beri as the result of being fed exclusively upon polished rice could be "cured" with any preparation which would cause animals to grow when it was added to a diet of purified food-stuffs containing a growth-promoting fat. See Chart III.

McCollum and Davis in 1914 followed the method of Funk and Suzuki of employing alcoholic extracts of various natural foods. They added the alcohol-soluble matter to the standard mixture of protein (casein), starch (dextrinized), salts and butter fat, and became convinced that the substance which relieves polyneuritis in pigeons was always present in the preparations which rendered the diet of purified food-stuffs capable of promoting growth. An alcoholic extract of ether-extracted wheat germ was finally adopted as a source of this dietary factor in later investigations. Funk and his coworkers had previously shown that the curative substance is present in many natural foods (1915). Repeated experiments by McCollum and Davis showed that the inclusion of the alcoholic extract of wheat germ or of other food was not sufficient to induce growth *unless the butter fat or other similar fat was likewise added to the purified food mixture. Both the growth-promoting fat and the trace of unidentified substance in the alcoholic extract of wheat germ are necessary for the promotion of growth or the preservation of health* (1915).

Funk and Macallum found butter fat to be ineffective for the cure of polyneuritis in pigeons, and accordingly considered it

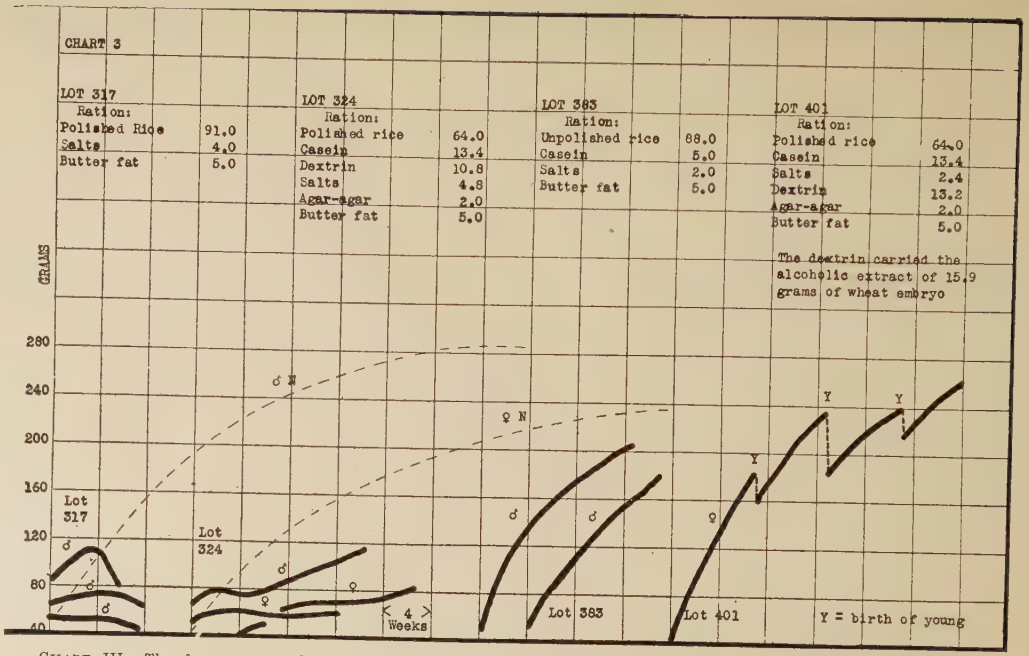


CHART III.—The four groups of curves illustrate the nature of the deficiencies of the polished rice kernel as a food for the rat. Lot 317 could not grow on rice, a complete salt mixture and butter fat because the anti-neuritic substance vitamin B was absent. Improvement in the protein by the addition of casein did not enable the animals to grow except to a very slight extent. Unpolished rice contains the germ which is rich in vitamin B, so the addition of the same supplements as in Lot 317 served to complete it and make a diet on which growth was rapid. Lot 401 differed essentially from 324 in that it contained the alcoholic extract of wheat embryo or germ. This supplied vitamin B, and therefore enabled the animals to grow, since all other deficiencies of polished rice were made good by the addition of a protein (casein), a complete mineral salt mixture and butter fat which contained vitamin A.

free from "vitamine." They fed young rats a diet of purified food-stuffs supplemented with a liberal amount of butter fat, and observed that the animals did not grow. Although this was the result of the lack of the antineuritic substance, no one was at that time clear as to the several factors which are necessary in the diet and they interpreted the data as evidence that butter fat possessed no growth-promoting properties (1915).

Observations on Lipin Extracts.—Stepp was unable to detect any special dietary property in butter fat. He showed that alcoholic extracts of certain natural foods, *e.g.*, egg yolk, bran, dry milk, possessed remarkable properties in that they completed from a nutritional standpoint his lipoid-free bread and milk diet. This is now readily understandable. His alcoholic extracts contained some of both the uncharacterized substances discussed above, whereas butter fat contains but one. The latter, without a supplementary source of a second dietary factor would permit of failure of the experimental animals.

Vitamin Hypothesis "In the Air" in 1914.—From what has been said of the experimental studies which ultimately led to the conception that there were necessary two uncharacterized dietary essentials in addition to the long recognized food principles in the diet of the rat, it will be appreciated that about 1914-15 there were suggestions of various kinds "in the air," and that the time had arrived when some of the several investigators working in the field would soon formulate on the basis of definite experimental evidence a working hypothesis concerning the essentials of an adequate diet. Thus Mendel, in his Harvey lecture, November, 1914, said, "It is not unlikely—to speak conservatively—that there are at least two 'determinants' in the nutrition of growth...Without this 'determinant'...the special components of butter fat or cod liver oil or egg fat induce only limited gains at best. Another 'determinant' is furnished by these natural fats. Either of the determinants may become 'curative,' both are essential for growth when the body's store of them (if such there be) becomes depleted. It is too early to attempt a tenable conclusion."

Essential Components of Adequate Diet.—As a result of these experiments McCollum and Davis formulated in 1915 their working hypothesis of what constitutes an adequate diet. They stated that the diet must contain, in addition to the long recognized dietary factors, viz., protein, a source of energy in the form of proteins, carbohydrates or fats; a suitable supply of

certain inorganic salts, and two as yet unidentified substances or groups of substances. One of these, vitamin A, is associated with certain fats, and is especially abundant in butter fat, egg yolk fats, cod liver oil, and the fats of the glandular organs such as the liver and kidney, but is absent, or present in but traces, in fats or oils of vegetable origin. The second substance, vitamin B, is never associated with fats or oils of either animal or vegetable origin. It is widely distributed in natural foods. It can be isolated in a concentrated form but has not been prepared in a state of purity. The vitamin B is soluble in water and dilute alcohol. These extracts always contain the substance which cures polyneuritis in birds.

This hypothesis seemed to contain all that could be said about the needs of the rat for dietary essentials hitherto unappreciated at that time. Later it became evident through the studies of Holst, Chick, Mendel, and others, that man and the guinea pig require a third substance, now known as vitamin C, which is not necessary in the nutrition of the rat. Studies on bone growth as influenced by diet have demonstrated that another unidentified factor is necessary for the normal development of bone, the vitamin D (see Chapter XX). Evidence has also been adduced which supports the view that a specific substance called by Evans and Bishop "Vitamin X" is necessary for the normal reproduction of the rat, and also that another specific substance derived from the food is essential for the successful performance of lactation in this species. Benedict and Sugiura (1919) have also reported observations which they interpreted to indicate that a diet can be adequate for the production of young and for growth after the eyes are opened but inadequate for the proper production of milk. These results suggest the necessity of some specific principle for milk secretion.

Nomenclature of Vitamins.—The nomenclature of the newly discovered nutrient principles is more or less in a state of confusion. Stepp (1909), who has the merit of having first established the indispensability of certain alcohol-ether soluble substances in the diet, regarded them as lipins. Funk (1913) designated as "vitamines" a hypothetical group of protective substances of unknown nature. Hopkins (1912), after discovering the remarkable growth-promoting effects of small additions of milk to a diet of purified food-stuffs, termed them "accessory" food substances.

Bottomley (1914) designated as auximones a group of sub-

stances which he believed stimulate plant growth. Wildiers in 1901, long before the formation of a conception of the vitamin hypothesis, observed that when a synthetic medium containing only well-defined substances was inoculated with yeast no growth occurred unless the inoculation was a large one. He explained the phenomenon by supposing that the yeast required some unknown substance for its development which was not present in a synthetic medium but was found in beer wort and in all growing cultures of yeast. He gave the name "Bios" to this substance and investigated its properties. The oldest name x-Saure was proposed by Hulshoff-Pol to designate the antineuritic substance (1910). Suzuki and Shimamura called the substance curative for beri-beri "Aberisaure." Funk gave it the name "vitamine." Various commercial preparations have received such names as Oryzanin, Torulin, Orypan, Antiberiberin, Oridin, Sitacoid, Eutonin, and Nutramin.

When it was apparent that there were two unidentified dietary factors necessary in the nutrition of the rat, one soluble in fats, the other never associated with fats but easily dissolved out of natural foods by water or dilute alcohol, it became necessary to differentiate between them by names which would characterize them. None of the terms in use seemed desirable, so McCollum and Kennedy (1916) suggested the provisional use of algebraic terms, using a prefix designating characteristic solubility. They proposed the term fat-soluble A and water-soluble B respectively to designate them. These terms found widespread acceptance.

Upon the establishment of the existence of an antiscorbutic substance Drummond (1919) designated it water-soluble C. He later (1920) suggested that the spelling of vitamine be changed to vitamin, and that this term be adopted to designate the entire group of chemically uncharacterized dietary essentials. This is in accord with the nomenclature of the hormones and the alkaloids, and indicates nothing of their chemical natures.

Gradually the terms "Vitamin A," "Vitamin B" and "Vitamin C" have come into general use and seem to be the most desirable from the standpoint of brevity. The demonstration of still other nutrient principles belonging to this class makes it imperative that a system of nomenclature be adopted which not only distinguishes readily the members whose existence is well established, but which easily provides for the naming of any new nutrient principle of this class. In 1922 a substance which

exerts a profound influence on bone development under certain conditions of faulty diet was shown to exist and received the name of Vitamin D. Provisionally Evans and Bishop designated as Vitamin X rather than Vitamin E a substance which their studies indicate to be essential for reproduction. Sure (1923-24) has extended the investigations of Evans and Bishop, and confirms their findings. He calls the substance X Vitamin E.

CHAPTER III

THE EARLIER VIEWS OF NUTRITION PROBLEMS

Beaumont's Views on Digestion.—Although food constitutes the chief item in the expense of living among the wage-earning class, and is more intimately concerned with the promotion of health and strength than are clothing, shelter and climate, it is only very recently that any serious thought has been given to the nature of food, and to the processes which it undergoes in being utilized for the bodily upkeep. Early man used the food he found available. Latitude, climate, soil and proximity to large bodies of water determined his dietary habits. Common observation showed that people in different regions of the world lived on diets of widely different character, yet without any markedly different success in physical development. Indifference to the nature of the food supply, with the exception of palatability, was therefore natural, since there was no obvious evidence that the character of the diet had anything to do with well-being.

The paucity of our knowledge concerning nutrition that existed toward the middle of the nineteenth century is well illustrated by the views expressed by Beaumont in his book "Physiology and Experiments," published about 1832. He had the good fortune to study the processes of digestion with the hunter, Alexis St. Martin, who had a fistulous opening into the stomach as the result of a gunshot wound. Beaumont collected gastric juice from his subject's stomach and studied its effect on various foods. He also introduced foods into the stomach and observed through the opening the behavior of this organ during digestion. He believed that the views of others to the effect that there were various kinds of nutrient substances were erroneous. In his opinion there was but one kind of food or "aliment" as he termed it. He believed this was present in all foods, and was simply dissolved out by the action of the gastric secretion. Beaumont was one of the most progressive investigators of his time.

The chemistry of both inorganic and organic substances advanced with great rapidity during the nineteenth century. Among the facts which were established was that foods contained proteins, carbohydrates and fats, thus disproving the view that there was but "one kind of aliment." It is the oxidation of these food substances that yields the energy which keeps the body warm, and enables it to do mechanical work. The laws governing energy metabolism were next developed.

Lavoisier Placed Nutrition on Scientific Foundation.—The modern era of the science of nutrition may be said to have been introduced by the famous French scientist, Lavoisier, in 1780. He was the first to apply the thermometer and the balance to the study of the chemical changes taking place in the living body. He established the fundamental fact that the production of heat involved the combination of the oxygen of the air with the substances taken into the body as food, and was in fact a process of combustion. He discovered that the temperature of the air had a pronounced influence on the rate at which substances in the body were oxidized. Oxidation is slow at temperatures which we find comfortable with light clothing, and is greatly accelerated by cold. He found exercise to stimulate combustion in the body, and he also observed that during work it might rise to several times the resting metabolism. These fundamental facts established by Lavoisier have formed the subjects of numerous investigations, which greatly increased our knowledge of detail in this important field.

About 1842 Joule described experimental data establishing the mechanical equivalent of heat. Heat is measured in terms of the small calorie, which is the amount of heat necessary to raise the temperature of 1 c.c. of water 1 degree Centigrade. The calorie used in dietetics is the large or kilo calorie which represents the amount of heat required to raise 1 kilogramm (2.2 pounds) of water 1 degree Centigrade or 1 pound of water approximately 4 degrees Fahrenheit. The rate at which energy metabolism varies with the activity of the individual, and in the absence of suitable clothing with the temperature, is the most spectacular phase of the metabolic processes. A man at rest may need about 1,600 calories a day in the form of suitable food, in order to cover his energy requirements. The same man at very severe labor requires 10,000 calories a day. It is not surprising that this obviously important phase of metabolism was early studied in great detail.

Studies on Energy Metabolism.—Pettenkofer and Voit (1866) in Munich began the publication of their elaborate studies on energy metabolism. With the aid of a respiration calorimeter they measured accurately the amount of oxygen absorbed, the carbon dioxid and the water in the products of respiration, and the heat given off by the body under various conditions. They introduced into their experimental work the idea suggested by Liebig in 1842, viz., that the nitrogen eliminated in the urine could be made a measure of the amount of protein destroyed in metabolism, since protein is the one prominent food-stuff which contains this element. Pettenkofer and Voit studied the metabolism of fasting men and dogs, and of the same subjects when taking different kinds and amounts of food. From the magnitude of the "respiratory quotient," which is the figure obtained by dividing the volume of carbon dioxid eliminated by the volume of oxygen consumed in respiration $\frac{(CO_2)}{(O_2)}$ it was possible to decide what kind of food was being oxidized as a source of energy in the body. When carbohydrate is burned the quotient is 1. When fat is burned it is 0.7, and when protein is burned the figure is approximately 0.8. They found that a dog could be maintained in nitrogen and energy equilibrium during a period of a few days on a diet of protein alone (muscle tissue).*

Specific Dynamic Action of Food-stuffs.—The early studies relating to energy metabolism led to the discovery that the protein of the food differs in one marked respect from either carbohydrate or fat, viz., its great stimulating effect upon metabolic processes as measured by the rate of oxidation. This is illustrated by the following type of experiment: If the rate of energy metabolism of a dog when it has been for some hours without food is carefully measured, it will be found that this remains constant within narrow limits so long as the animal remains at rest. If now it is fed liberally with either fat or carbohydrate and the observations on its energy metabolism are continued, there is observed a slight increase in metabolism which is the direct result of the utilization of these foods. If on the other hand, a liberal portion of protein-rich food, such as meat, is fed to the animal instead of the non-nitrogenous

* For a detailed history of this important phase of nutrition, the reader is referred to "The Science of Nutrition," Graham Lusk, 3rd Ed., Philadelphia, 1917; and to "The Chemistry of Life," L. B. Mendel, New Haven, 1923.

nutrients, there is a surprising acceleration of the metabolic processes, attended with a marked increase in energy set free within the tissues. This was first shown by Bidder and Schmidt in 1852. Their experimental animal was a cat. During a certain interval of time the animal used 50.18 gm. of oxygen and eliminated from its lungs 53.52 gm. of carbon dioxid. The animal was then allowed to eat all the meat it would consume and the observations continued. During a similar interval the cat used 103.84 gm. of oxygen and respired 113.52 gm. of carbon dioxid.

The remarkable researches of Voit, Pettenkofer, Rubner, Atwater, Lusk, Zuntz and Benedict on the metabolism of matter and energy in the body attracted deserved attention. For years this kind of study occupied a large area in the field of vision of students of nutrition. The researches were extended to the study of some of the finer problems of metabolism. It was shown that carbohydrate can be converted into fat by the tissues, and that a part of the carbon which the protein molecule contains can be converted in the body into glucose. These investigations shed much light on such conditions of perverted metabolism as prevent the oxidation of sugar in the tissues (diabetes). In the light of the new developments in the chemistry of proteins and their digestion products, the amino-acids, Lusk finally explained in great measure the cause of the specific dynamic action of proteins (1915). Lusk (1922) says in the presence of an abundant quantity of oxidizable fragments of carbohydrate metabolism the heat production is raised to a high level. Definite affinities for carbohydrate consumption are satisfied which are not involved when the extra supply of glucose is being continually depleted under the regulatory control of the liver. The production of increased heat after carbohydrate ingestion may be termed the metabolism of carbohydrate plethora. The same conclusion is reached regarding fat as regarding carbohydrate: That in the presence of an amplitude of fat particles there is a metabolism of fat plethora due to the utilization of fat by special fat receptive cellular affinities. The specific dynamic action of protein consists in a specific chemical stimulus of the cellular protoplasm which is independent of the oxidation of the material through which the stimulus is applied. It may be termed the metabolism of animo-acid stimulation. A theory of metabolism is stated which is a modified form of one enunciated by Rubner: "One may conclude that the influence of

food ingestion upon the basal metabolism of the quiet resting cell may be upon three independent mechanisms within the cells: (a) a mechanism which is receptive to a chemical stimulus derived from such amino-acids as glycine and alanine; (b) a mechanism of carbohydrate plethora which allows metabolism of carbohydrate up to certain limits imposed by self-regulation, (c) a mechanism capable of receiving power from that quota of fat which when in excess increases the heat production of the cell."

Chemical Analysis of a Food-stuff.—After 1900 the view began to develop that proteins differed markedly in their chemical composition. Previous to that time it was believed by those interested in the nutrition of man and animals that the results of a chemical analysis were of the greatest importance in showing the nutritive value of any food, and on the basis of such analysis advice was given as to the manner in which foods should be combined. The technical methods in use at that time were first described in 1864 by Henneberg, and were adopted by the German Official Agricultural Chemists, and afterwards used by food chemists all over the world.

The food analysis consisted of the following determinations: The nitrogen content, from which the protein was calculated by multiplying the value found by 6.25, on the assumption that all proteins contained approximately 16 per cent of nitrogen; the fat, by extracting the food with some solvent, usually ether, and weighing the amount of substance which was extracted from a known amount of food; the "crude fiber," or cellulose, by treating a sample of the food with a solution of acid and then with one of alkali sufficiently strong to dissolve all components except the more resistant forms of cellulose, and a small amount of mineral matter which this cellulose retained; the ash, or mineral matter, by burning a weighed sample and subsequently weighing the residue left after incineration. The analysis was made on a sample of food which was carefully dried to constant weight in order to free it from moisture. The sum of the protein, fat, ash and fiber was then subtracted from 100 to give a figure which was designated "nitrogen-free extract." This was assumed in calculations used in estimating the amount of food necessary to meet the requirements of an individual as being essentially a measure of the amount of utilizable carbohydrate contained in the food. This in outline is the chemical analysis of a food. It has been shown later that the biological method of analysis

is very essential to complete our knowledge of the nutritive value of foods.

By 1900 very careful determinations had been made of the amount of heat liberated when a definite weight of the food is burned. This was known as the calorific or fuel value. Numerous determinations had been made of the amount of energy required per day for a man of ordinary size when at rest and when doing several kinds of work representative of moderate and more severe kinds of labor. The illustration most widely used by writers on nutrition was that of comparing the body to an engine, in which fuel (food) was burned and by which mechanical work was performed, with the liberation of a certain amount of waste energy in the form of heat. The adjustment of the supply of calories, or energy units of food, to the needs of the individual, and the amount of protein necessary to make good the "wear and tear" of the tissues, which is an unavoidable result of the life processes, were considered as the two factors of prime importance in the planning of the diet.

Elements of Weakness in Standard Food Analysis.—The standard food analysis regarded of so great importance had a number of shortcomings as a method of determining the nutritive value of a food. By means of it the great differences in the make-up of the proteins, which give them very unlike value in nutrition, were not revealed. Protein from one source was assumed to be as good as that from another. Beans and peas, for example, contain about 23 per cent of protein, and compare favorably in this constituent with lean meat. These legume seeds were regarded for many years as essentially the equivalent of meat as a source of protein. They were sometimes spoken of as "the poor man's meat." The proteins of these seeds have peculiarities in their composition which make them of relatively low value in nutrition when they serve as the sole source of protein in the diet, or when they are combined with some of our most important food grains. Meat proteins are decidedly superior to them as supplements to most of the proteins of our vegetable foods. This was only brought to light by later investigations.

The analysis of foods for their carbohydrate content was not effective as a means of arriving at a decision as to the energy value of this portion of their substance. Man and animals can use starch and sugars as a source of energy, but not cellulose or hemicelluloses. The best method for differentiating between the

several types of carbohydrates was that based on the ease with which they are converted into glucose or other simple sugars by the action of acid of certain concentration. The true celluloses, of which paper is made, are not dissolved by the reagents used in food analysis, and could be filtered off, washed, dried and weighed. There are many kinds of vegetable foods which contain considerable amounts of hemicelluloses, a class of carbohydrates which are easily converted by the action of acid into simple, soluble sugars in the process of analysis. Accordingly, these were estimated as part of the fraction called "nitrogen-free extract," and were, in the absence of specific data as to their digestibility, estimated in calculations of dietaries, as the equivalent of starch or sugar.

Hemicelluloses are not acted upon by the digestive secretions of man or animals. They have, therefore, no food value except that imparted to them through the agency of certain microorganisms inhabiting the digestive tract. These organisms may bring about their fermentation with the production of such organic substances as acids and alcohols as intermediary products. These may be absorbed and utilized by the body as a source of energy for mechanical work or for heat production.

The method for the estimation of fat in food-stuffs was fairly satisfactory, but the materials weighed as fat always contain waxes, cholesterol, chlorophyll, etc. These, with the possible exception of chlorophyll, have no food value, and are inert and chance components of the diet. The fats and carbohydrates were regarded as essentially sources of energy for work or for heat production, and were rightly considered as being able to replace each other in the diet in isodynamic quantities. One gram of carbohydrate or of protein has a caloric value of about 4.1, while 1 gm. of fat has about twice this fuel value.

The mineral content of a food-stuff was determined in the standard method of analysis by burning a weighed sample in a dish of known weight. After the organic matter was all destroyed the dish was cooled in a dry atmosphere and re-weighed. The difference between the weight of the dish and the dish plus the ash gave the weight of the mineral matter. Little significance was attached to the inorganic content of foods until recent years. It was assumed that, since all the elements which are required by the body are found in all foods, the amounts might vary considerably and still meet the needs of the body. In special cases the deficiency of a food in a particular element was so pro-

nounced that it attracted attention. Thus milk is especially poor in iron, and since this element is a constituent of the hemoglobin of the blood it was early recognized that a young child should not be too long confined to an exclusive milk diet, but should be given some food which would supplement milk in this respect.

Proportions Among Mineral Elements in Food Long Regarded as of Little Significance.—It was not possible to determine the adequacy of the content of our common foods in any of the inorganic elements until exact studies had been made to determine the efficiency of the animal body in absorbing and in conserving its supply. The variations in the inorganic content of the blood in health and disease are so small as to escape observation except by the most modern and refined methods. Yet these variations are of great significance to the welfare of the individual. Unwarranted assumptions were made by the earlier students of nutrition concerning the ability of the intestines to absorb mineral salts in a selective way so as to lead to utilization with high efficiency of those elements present in the food in small amounts. The capacity of the kidney to hold back those elements which are present in the food in minimal amounts and therefore reach the blood in amounts below the optimum was overrated. The efficiency of the kidneys in preventing the passage of inorganic salts into the urine along with the waste products of metabolism is not so great as to protect the body from losses which may be injurious to it when the food contains as little of such salts as those of calcium, phosphorus, sodium and chlorin as are contained in some of our most important cereal grains, tubers and meats. There are well-defined limits to the degree to which the intake of these salts can be restricted without causing damage.

Digestion Experiments.—It was fully appreciated many years ago by students of nutrition that the food analysis had its serious limitations. The belief prevailed that the way to eliminate the defects of chemical technic was to supplement the data obtained by chemical methods with information secured by actual trials to determine the digestibility of foods. The subject and the diet having been decided upon, samples of the food to be eaten were carefully analyzed to determine their content of protein, fat, ash, moisture and "nitrogen-free extract." The period of the experimental trial varied considerably, but in most cases it covered three days. It was customary to keep the subject on a milk diet during a fore period, so that the feces derived from the ex-

perimental diet would be different in appearance and could be accurately separated. The chemical constituents of the ingested food minus the amounts of each which appeared in the fecal residues afforded the data from which the digestibility of the protein, fat and carbohydrate were calculated.

There were some errors in this procedure. In addition to the food substances which escaped digestion, a part of the nitrogen and salts eliminated from the intestine were derived from the secretions of the digestive glands, and from the cellular elements which came from the intestinal mucosa. The quantity of secretions eliminated from the tract depended in great measure on the content of indigestible cellulose in the food. This acted as a sponge, and retained liquids so that they could not be reabsorbed. An animal which is fed a diet containing just sufficient protein to meet its needs, may easily be made to suffer a nitrogen deficit by including in its food supply a liberal amount of cellulose or other indigestible carbohydrate such as are abundant in many vegetables.

In interpreting the results of digestion experiments "utilization" of the several components of the food was assumed to be measured by the extent to which they disappeared from the digestive tract. That such an interpretation is not always justified is readily understood. A diet of cereal grains will be well digested and absorbed, but the substances which reach the blood and tissues are not a satisfactory pabulum for the nutrition of the body. Some complexes which are indispensable to the body are present in too small amounts to admit of the utilization of the other useful ones which are abundant. The latter cannot serve a useful purpose unless they can be used to form definite structures. Such a food supply may be well absorbed but not assimilated. Utilization is an unfortunate choice of a term in this sense for it is liable to be misinterpreted. Knowledge of the digestibility of food-stuffs is of value to students of nutrition, but the idea of quality in the array of digestion products must not be lost sight of.

It will be appreciated in the light of what has been said that the chemical analysis of a food, and knowledge concerning its digestibility, even when its palatability is satisfactory, do not give any information concerning its physiological effects. These can be discovered only by observations on the well-being of men or animals restricted *during considerable periods of time* to a definite type of diet. It was assumed for a long time, and

without any experimental evidence in support of the assumption, that any diet which complied with the composition of ordinary combinations of foods which experience had shown to be palatable and wholesome components of the diet, and was so constituted as to furnish the necessary amount of protein and of energy in digestible form, would promote growth and prolong well-being if available in sufficient quantities. This popular conception we now know to be far from true.

Study of Supplementary Foods in Animal Production.—It gradually became the custom in agricultural experiment stations to compare the effects of certain combinations of foods on the growth of young animals, and on milk production in cows. These experiments showed plainly that all rations of similar chemical composition are not alike in their physiological effects. In no instance was it possible, however, because of lack of knowledge of the several factors which enter into the normal diet, and because of the faulty planning and conduct of many of the experiments, to interpret the nature of the causes which determine their outcome. These belonged to the type of *immediate utility* experiment and were fostered by agricultural experiment stations because of their practical nature. They were of great economic value since they showed clearly that certain combinations of foods were either poor, good or excellent for the nutrition of animals. The experiments were not, because of the nature of the food-stuffs employed, and because of the simple mixtures which were monotonously fed to the animals, of such a nature as to make possible deductions applicable to problems of human nutrition. Much of this experimental work was done by men with little scientific training. The experiments were therefore poorly planned and controlled, with the result that confusion prevailed. Instances were not infrequent where the title of a paper would lead one to believe that the experiments were designed to determine the efficiency with which a certain protein-rich concentrate supplemented each of several cereal foods in the nutrition of growing swine, but a careful inspection would show that the ration described was fed to animals allowed to run on a pasture, and eating freely of a forage plant, or were given such a skim milk supply as the dairy department afforded. No account was taken of these factors in the interpretation of the results.

In 1900 nutrition studies made for the purpose of solving the problems of economic animal production had developed the con-

ception of the specific effects of different foods on the development of the body during growth, and for the performance of work or the production of milk, wool, etc. The skilled animal husbandryman was distinctly in advance of the human physiologist or the medical man in his knowledge of nutrition from the practical standpoint. Atwater, who represented the most advanced thought of his time, wrote in 1895 his "Chemistry and Economy of Food." This book clearly reflects his view that if we knew the chemical composition of all the food-stuffs entering into the human diet, their digestibility, energy values, and the energy requirements of an individual at rest and when doing various kinds of work, we should have all the necessary data to enable us to plan a highly satisfactory nutritive regimen. The well-balanced diet was one in which the proportions among the several recognized nutrients corresponded with the standards adopted as the result of statistical studies of what people in different circumstances and in different localities were selecting for their food supply when guided by the appetite and by expenditure.

Study of Food Requirements by Statistical Methods.—These dietary studies were all made after the plan introduced by Voit in Germany. Atwater made numerous studies of American dietaries. Much interest was manifested by the public in these results and others took up similar investigations, so that we have available the diets of people in all parts of the country and in all walks of life during the period from 1890 to 1900. The diets of Alabama negroes, Mexican laborers in New Mexico, students in boarding clubs, families of faculty members in several universities, Maine lumbermen, foreign families of various nationalities as well as workingmen's and professional men's households, were subjected to scientific scrutiny. The quality of any dietary was evaluated by comparison with the standards adopted by Atwater and Voit for energy and protein. This great body of studies were for the most part published in bulletins of the United States Department of Agriculture. Their collection represents an epoch in the development of knowledge of human nutrition.

Mistaken Idea in Economy of Foods.—When our knowledge of nutrition had advanced to this point it was natural that the idea of economy in food should attract a great deal of attention, because on the average half or more of the family income is spent for food. In the household of a wage-earner food is the largest single item in the family budget. Atwater made calculations concerning the amounts of protein, fat, carbohydrates and

fuel values which could be purchased for twenty-five cents. These results made it clear that at that time (1884) this sum in Massachusetts would buy one-sixth of a pound of protein, one-fifth of a pound of fat, and 1,120 calories of energy if expended for sirloin steak. The same amount spent for oysters at 50 cents a quart purchased one ounce of protein, two ounces of total nutrients and 230 calories of energy. In buying wheat flour at seven dollars a barrel the twenty-five cents would pay for six and a quarter pounds of nutrients containing eight-tenths of a pound of protein and 11,755 calories of energy. Since it was accepted that the quality of each of the nutrients from each of these foods common in the American diet was essentially the same, the logical course seemed to be to buy the cheaper articles when economy was essential. There was no fear that any diet would prove unsatisfactory provided it contained sufficient protein and sufficient available energy.

It was pointed out by Atwater that the price of food was not regulated solely by its nutritive value, but that agreeableness to the palate or to the fancy was a factor of great significance in determining the market price. There was no appreciation of peculiar worth of certain foods as supplements to others until after the year 1900. Nor was there any appreciation of the difference in quality of foods such as we now fully recognize. The astonishing fact that certain lists of common foods do not maintain satisfactory nutrition even though they have collectively a chemical composition exactly similar to those of another list which is satisfactory for the promotion of nutritive well-being did not become appreciated until about 1915.

Physiological Economy in Nutrition.—It is the great merit of Professor Chittenden of Yale University to have introduced into nutrition studies the idea of physiological economy in nutrition (1904-07). Numerous dietary studies in Europe and America had shown fairly accurately what the people in several countries were eating. The amounts and the chemical composition of the foods had been carefully determined. The most notable students of this subject were Voit in Munich, and Atwater in America. Voit came to the conclusion that an adult man weighing 70-75 kilos and doing moderate work requires a diet containing 3,000 calories of energy. He believed that it should furnish 118 gm. of protein daily, 56 gm. of fat and 500 gm. of carbohydrate. Atwater's standard called for 120 gm. of protein. These standards were based upon statistical studies of large numbers

of people. It was assumed that appetite and instinct were safe guides to the physiological needs of the body.

These standards were derived through studies of the actual consumption of food by families in various walks of life. Those who accepted them as the amounts of the various food components which the body requires under specific conditions, accepted the view that man, when in a position to select his food from a suitable variety such as exists in times of relative plenty and moderate values, would take under the guidance of the appetite such amounts of food as are best suited to his bodily needs. Chittenden was the first to seriously question this doctrine of instinctive selection. He applied what seemed at the time to be a crucial test for the trustworthiness of this doctrine. He restricted men over a period of nine months to diets, the protein content of which fell far below the amount which physiologists generally had accepted as the actual need.

Chittenden began cautiously experimenting on himself to discover how low he could reduce the protein in his diet without interfering with his well-being. To his surprise he remained in nitrogen equilibrium and in improved physical condition on a diet which contained only sufficient nitrogen of a digestible and absorbable nature to make the nitrogen content of the urine average 5.699 grams daily over a period of nearly nine months. This corresponds to about 36.72 grams of digestible protein. Chittenden felt convinced that this reduction of the intake of protein to approximately one-third of the accepted standard requirement for the human body resulted in marked improvement in physiological well-being. He determined to test this question on a scale which would afford convincing evidence, in order that mankind might profit by what was believed to be a great discovery, viz., that physiological economy in nutrition is a matter of great importance for the maintenance of strength and vigor.

The reasoning by means of which the good results of this abstemious diet, and especially of the low protein intake, were accounted for is interesting indeed. A diminished intake of protein leads to a decreased formation of crystalline waste products, such as acid potassium phosphate, uric acid and the purin bases and other end-products of nitrogen metabolism. These are in part responsible for the sensation of fatigue when they accumulate in the blood. The energy of work comes not from the degradation of muscle substance. It comes from the combustion

of non-nitrogenous food-stuffs such as sugar and fat, and it is not, therefore, necessary to take large amounts of protein food for the purpose of replacing the wear and tear quota, for the latter is but of small magnitude even in severe work. Protein stimulates body metabolism in general, and hence will lead, when taken in excessive amounts, to a rate of metabolism far in excess of the normal amount which results from a diet lower in this factor. This augmentation in the metabolism of the body tissues leads to a waste of energy-yielding substances which might otherwise be conserved for useful expenditure.

It was noted by Chittenden that while on the low protein diet there was not a single day during the nine months when his urine was turbid, whereas on a rich protein diet it was necessary to take a large amount of water in order to prevent the kidneys from becoming clogged. It was reasoned that this low protein regimen should relieve the kidneys of a burden and would tend to aid them in maintaining their functional capacity unimpaired.

In order to demonstrate in a large way the importance of his new views, Chittenden secured the co-operation of a group of his colleagues in the faculty of Yale University and a group of volunteers from among the students. These men were allowed a free choice of food, but they conscientiously adhered during the period to the regulations which were imposed of selecting foods which were poor in protein. A group of soldiers of the United States Army were detailed under the command of a sergeant to participate in the experiment. The diet afforded to these men was similar to that taken by those who volunteered to subsist on a diet low in protein and of a somewhat abstemious character.

In the case of the faculty and student groups the diet chosen contained little or no meat, and this automatically tended to lower the protein intake to a decidedly low level. In all cases there was free choice of food in these two groups.

There was no indication that the men who participated in this experiment were not in excellent physical condition at the end of the nine months during which they were nourished on an abstemious although somewhat more liberal diet both in energy and protein than that which Chittenden had taken with apparent benefit. The conclusion was drawn that the general standards of food consumption of people were faulty. The faults in the ordinary diet lay in the excessive consumption of food and especially of protein. The view seemed sustained that the body

is better nourished when the amount of food metabolized closely corresponds with the minimum which is necessary to cover its requirements. This better nourishment was held to be due to the relief of the digestive organs and the organs concerned with the transformation of protein food. The liver, as well as the organs of excretion, the kidneys in particular, were relieved from an excessive burden to which the ordinary eating habits of man subject them.

There can be no doubt that the diets which these men took did not lead to any observable deterioration in their physical well-being. All practically maintained their body weights, or at least lost only such amounts as would be anticipated as the result of the physical exercises some of them indulged in during the test. Their strength increased during the experiment where records were taken and all were said by their observers to be in a normal and healthy condition at the end of the experiment.

Parsimony in Nutrition.—Chittenden's conclusions were not accepted by many students of human nutrition. A critical discussion of his experimental data and conclusions was presented by Benedict (1906). He interpreted the digestion data obtained with the soldiers with whom Chittenden experimented as indicating that the low protein intake had influenced adversely the absorption of protein from the alimentary tract. He further called attention to the fact that animals fed low protein diets do not thrive so well as on more liberal quantities. Benedict, and also Crichton-Browne (1909) pointed out that dietary studies all over the world had shown that in civilized communities where productive power, enterprise and civilization are at their highest, man has instinctively and independently selected liberal rather than small quantities of protein.

Studies in Under-Nutrition.—In 1917, when there was a food shortage brought about as the result of the World War, Benedict conducted an experiment on twelve men, students of the Y. M. C. A. College at Springfield, Mass., to see what the effects would be of rapidly reducing the body weight by about 10 per cent by a sharp reduction in the amount of food ingested. This was accomplished in 4 to 6 weeks. Thereafter the food consumption was so regulated as to maintain the weight at the new level. Each man lost approximately 150 gm. of nitrogen, equivalent to about a kilogram of protein. Their metabolism was reduced, therefore, to a distinctly lower level than normal during the greater part of a school year. From the results of his observa-

tions he was led to modify his former view and to become a convert to the idea that the abstemious diet best promotes physical well-being (1918).

Experiments Not Planned to Give Conclusive Results.—The question naturally arises: How far are we justified in drawing conclusions from the data furnished by these experiments as to whether or not this is a satisfactory regimen to which man may safely adhere throughout the period of adult life? It must be admitted that in the light of the new viewpoints which have been gained by animal experimentation during the past decade, these results on men do not seem sufficiently convincing to warrant any conclusions relative to the most important of all questions which arise in the life of the individual, *viz.*, that of maintaining to a late period in life the full vigor of youth.

These experiments on men covered approximately 1 per cent of the normal span of life for individuals of their age. It has been abundantly demonstrated on animals that diets which fall below the standard of quality necessary to maintain life and vigor over a very great fraction of the span of life of which the species is capable of living, may cause no noticeable deterioration in the physical condition, during even 5 per cent of the normal expectation of life. In order to produce noticeable effects by faulty diet during 4 or 5 per cent of the average life, it is necessary that the faults shall be relatively severe. Yet diets which are just good enough to permit a young animal to develop in what appears to be an approximately normal manner, may cause early ageing, and instability of the nervous system. We know that while growth is still in progress, or after it has ceased, the adherence for even brief periods of a few days or weeks to a diet which is unsatisfactory in some degree, leads to a deviation from the normal histological structure of the osseous, the nervous or the circulatory tissues, depending upon the nature and extent of the faults in the food. Such abnormal modification of tissue structure would ordinarily escape observation even to-day, and certainly a few years ago.

Effects of Faulty Diets on Life Histories of Animals.—Let us illustrate the principle we have in mind by the results of animal experimentation. In the author's laboratory a large group of rats were restricted to diets which were faulty in different degrees with respect to the quality of their protein content. The experiments included the period of growth, and of adult life at least to the point of marked development of senile characters.

The fertility and success with the nursing of young, the stability or instability of the nervous system as shown by the indifference to being handled, which is characteristic of the well-nourished rat, or the apprehensiveness and timidity of many malnourished ones, were carefully observed. Successive generations of the same families, where young were reared, were maintained on the family diet. These experiments will be discussed more in detail in a later chapter. It may be stated here that the deviation of the quality of the protein moiety of the diet even in slight degrees from the optimum, exerts a profound influence on the *life history* of the animals and on the *functioning of the nervous system.*

It may be added that the faults of a diet need be no more pronounced than are encountered in a mixture consisting of cereal grains, legume seeds, tubers, fleshy roots and muscle meats, to produce profound physical deterioration when such a regimen is adhered to over any considerable period.

Experiments on Man Less Than Five Per Cent of Span of Life.—The experiments of Chittenden and of Benedict covered but approximately 1 per cent of the expectation of life of the young men who served as subjects. They can scarcely be expected to be used as a basis of deductions of a far-reaching nature concerning the dietary practices which man may safely adhere to throughout long periods. Our conclusions as to what is safe or unsafe must be derived from tests of special types of diets on animals. The life history must be carefully observed, and the vitality of the offspring carefully compared with a satisfactory standard of excellence. The correlation of these results with the experience of man in various parts of the world, where groups are found whose dietary habits are of peculiar and restricted types, may be made. Such data will be set forth in later chapters of this book.

Optimal a Better Goal Than Normal in Nutrition.—The studies of McCollum and Simmonds have introduced into the science of nutrition the idea that the word "normal" is too indefinite a term. It is less satisfactory than "optimal" to designate the standard of excellence toward which we should strive in respect to physical development. They have emphasized that our standards of what constitutes normality are based upon common observation rather than upon the best possible achievement as illustrated by the best specimens in the population. These investigators also pointed out that it is unsafe to judge

by the appearance or feelings of a man or animal under special conditions during a relatively small fraction of life, especially that part in which recuperative power and endurance are greatest, that a dietary regimen which has proven unsafe for the promotion of vigor and health in animals, is safe for man. Physical deterioration may not be recognized until it is pronounced. It is important to appreciate that in the aggregate it is borderline states of malnutrition, characterized by a condition of nutritional instability, that is the greatest menace to the individual when his health and efficiency over the life period is considered.

With this attitude toward the subject of our discussion, we may now turn to some of the more technical phases of nutrition investigations. The results of these have served as a basis of practical conclusions of great value for the health of man.

CHAPTER IV

THE PERIOD OF ORIENTATION IN NUTRITION STUDIES

Osborne and Mendel's Comparative Studies on Proteins.—Since 1909 numerous experiments have been conducted with a view to determining the relative nutritive values of a large number of individual proteins. It has been pointed out in Chapter II that Osborne and Mendel first sought to make comparative studies of isolated proteins by adding them to a mixture of purified carbohydrates, fats, mineral salts and water, and invariably met with failure. After their failure with the diet composed entirely of purified food substances, every component of which was known, they began a series of studies with what they believed to be essentially the equivalent of a purified food mixture. This involved the inclusion in the diet of about 28 per cent of a product which they called "protein-free milk."

"Protein-free milk" was prepared by acidifying with acetic acid milk which had been freed from fat by centrifuging. The resulting whey, after the removal of the casein which flocks out on the addition of acid, was next heated to boiling to coagulate the lactalbumin. These are the principal proteins of milk. Their removal extracts about 93 per cent of the total nitrogen contained in the milk. The whey which was filtered from the lactalbumin precipitate was evaporated to dryness, leaving a yellowish residue consisting of milk sugar, most of the salts of the milk, and a certain amount of nitrogenous compounds for the separation of which no method is known. The final product which they included in their diets contained about 0.7 per cent of nitrogen. This would be equivalent, if it were all in the form of protein, or of its digestion products, to 4.37 per cent of the food mixture. It is well known that some urea, purins, creatin, and other simple nitrogenous substances which circulate in the blood are always found in milk. It is certain, however, that there is a considerable amount of nitrogen in "protein-free milk" in forms

yielding amino-acids in the body. Its property of enhancing certain incomplete proteins makes this view necessary.

Osborne and Mendel felt justified by the work of Munk (1893) in assuming that the 0.7 per cent of nitrogen in "protein-free milk" was not a disturbing factor in their experimental studies. Twenty years previously Munk sought by coagulation and by precipitation with various reagents, to determine the amount of "non-protein" nitrogen in milk. He arrived at the conclusion that one-fifteenth of the total nitrogen in milk is in forms other than protein. Osborne and Mendel accepted this figure and reasoned as follows: A certain amount of milk contains a known amount of nitrogen, a part of which is in the form of proteins and can be removed by the methods employed. On evaporating the resulting whey to dryness the "protein-free milk" which remains contains 0.7 per cent of nitrogen in forms not known. From the weight of the "protein-free milk" obtained the total nitrogen it contained was calculated. From this amount they subtracted one-fifteenth of the total nitrogen contained in the milk with which they started. The remainder was assumed to represent "protein" or nitrogen having a nutritive value. There is no evidence in Munk's data, or in any furnished by later investigators, to show that the one-fifteenth of the total nitrogen of milk which remained after the removal of the casein and lactalbumin, and which was disposed of by mathematical treatment of their analytical data, was not a source of amino-acids.

The diet used in their studies, containing 18 per cent of added protein in purified form, together with 28 per cent of "protein-free milk," would derive 93 per cent of its total nitrogen from the former and 7 per cent from the latter source. The biological evidence appears convincing that this 7 per cent of the nitrogen of their diets supplemented in a very effective manner certain proteins with which it was fed, and led in some instances to erroneous conclusions. Drummond (1916) commenting on these early studies said: "Osborne and Mendel have always been careful to utilize throughout their extensive researches proteins of a very high degree of purity, and yet they have gone to the other extreme in continuing to use in their diet 'protein-free milk.'"

Enzymes in Milk.—Babcock and Russell (1900) and Freudenreich (1900) demonstrated in milk the presence of protein digesting enzymes. The bacteria found in market milks are in many instances capable of digesting proteins. There is, there-

fore, abundant opportunity for milk used for the preparation of "protein-free milk" after it has been kept for an interval of 24 hours or more to contain partial digestion products of protein that would not be removable by acidifying or by coagulating. All such components would remain in the "protein-free milk."

Hijikata (1922) isolated and identified histidin, arginin, lysin, glycocoll and glutamic acid from fresh milk. It is known that these amino-acids circulate in the blood, but this is the first time they have been identified in milk.

Viale (1921) says amino-acids of milk (tryptophane and cystine) do not depend upon the presence of a tryptic enzyme, but are secreted by the mammary gland.

Limiting Amino-acids in Proteins.—From what has been said in Chapter III it will be clear that a protein which is of low biological value for growth because of the fact that one or more of the digestion products which it contains is present in too small an amount to make possible its efficient transformation into body proteins, may be greatly enhanced in growth-promoting value by the addition of the missing amino-acid or acids. This has been illustrated in the case of gelatin and for the meat digests prepared by Abderhalden (1913). It will be readily understood that when a series of purified proteins was fed to young animals, the remainder of the diet consisting of 28 per cent of "protein-free milk," together with non-nitrogenous nutrients similar to the following:

	per cent
Protein	18.0
"Protein-free milk"	28.2
Starch	20.8
Agar-agar	5.0
Fat	28.0

the amino-acids furnished by the "protein-free milk" may have improved the quality of some or all of the pure proteins. This would change the experimental results so as to make it unsafe to draw conclusions concerning their relative nutritive values when fed without such an amino-acid supplement.

Osborne and Mendel held the view that the amount of protein or its equivalent, resulting from the digestion of milk protein or directly secreted by the mammary gland, which was contained in their "protein-free milk," was too small to be a factor of significance in their experiments. They finally abandoned the use of the product in 1917, at which time they adopted

essentially the method of study which has been described in Chapter II.

Nitrogen Compounds in Protein-free Milk.—Some indication of the nutritive value of the nitrogenous compounds of "protein-free milk" is afforded by experiments carried out by McCollum and Davis (1915). They showed that young rats slowly lose weight when confined to a diet containing 1.5 per cent of protein and derived entirely from skim milk powder. The rats maintained weight over considerable periods when the amount of milk proteins was increased to 3 per cent of the food mixture. On the other hand, 1.5 per cent of milk protein supplemented with nitrogen equivalent to 1.5 per cent of protein derived from "protein-free milk" served to maintain the animals approximately as well as did 3 per cent from milk powder. From this observation they drew the conclusion that the nitrogen in "protein-free milk" has approximately the same nutritive value as that contained in milk proteins. This would not necessarily be true when it serves as the sole source of nitrogen in the diet, but only when combined with the proteins of milk in equivalent amount.

Kennedy (1919) states that amino-nitrogen determinations before and after hydrolysis and the nitrogen distribution indicate that either unprecipitated protein or peptides of considerable size are present in "protein-free milk." It seems evident from the data available that "protein-free milk" is capable of supplementing certain proteins because of the presence in it of bodies which can yield amino-acids.

Sure (1920) has shown that "protein-free milk" contains a total sulphur content of 0.2 per cent, the greater part of which is in organic form. His results suggest that "protein-free milk" contains either cystine or organically bound sulphur which the organism can transform into cystine. "Protein-free milk" gave a qualitative test for tyrosin.

Sure (1923) investigated the rôle of "protein-free milk" as a supplement to the total proteins in milk when casein and lactalbumin were introduced into the ration in relatively the same proportions in which they occur in milk. He found that lactalbumin has no supplemental value to the casein when the total proteins of milk are fed at 10 per cent of the diet. When "protein-free milk" was introduced into the ration to the extent of 28 per cent, replacing 4 per cent of salt mixture and 24 per cent of dextrin, it showed considerable supplementary value to the

total proteins of milk. Four-tenths per cent of cystine showed similar biological response; so the nature of the supplementary value of "protein-free milk" must be credited to the foreign nitrogen which it carries rather than to the superior value of the salt mixture. He says that "protein-free milk" has a supplemental value to the total proteins of milk at a 10 per cent plane of protein intake, which may be attributed either to cystin or to some other organic sulphur compound transformable into cystin.

There were three principal phases of protein nutrition which Osborne and Mendel discussed in the light of their data. The importance of their deductions is so great and the defects in their method so difficult for the inexperienced to appreciate, that a somewhat full discussion is warranted of the manner in which "protein-free milk" vitiated a considerable part of a research of a most comprehensive type. Three phases of protein metabolism will be discussed here, *viz.*, the possibility of the existence for the maintenance of the species of a synthetic capacity in the maternal organism, through the medium of the placenta and of the mammary gland, which can not be affected by other body tissues for the maintenance of the life of the individual; the comparative values of the individual proteins in growth; the protein requirements of the animal body in maintenance as contrasted with growth. These will be considered in the order enumerated.

Since the nitrogen contained in "protein-free milk" can act as a source of certain amino-acids, it is obvious that the true dietary value of any series of proteins which it is desired to compare cannot be secured when they are studied by the method involving the concomitant feeding of this material. The scope of the studies of Osborne and Mendel, and the importance of the deductions which have been drawn from them, are so great that a detailed exposition of the action of the nitrogenous constituents of "protein-free milk" in vitiating some of their results will be presented. This is best brought out by their studies of the nutritive value of gliadin, one of the principal proteins of the wheat kernel (1912). These are of fundamental importance because their interpretation involves the question, not only of the biological value of "protein-free milk" as a source of amino-acids, and its bearing on the recorded nutritive values of numerous proteins, but likewise on the problem of whether the processes of maintenance without growth, or the repair of tissue waste,

are of a lower order than are those of growth and require fewer amino-acids than the latter.

Lysin as Limiting Factor in Nutritive Value of Proteins.—Osborne and Mendel (1912) reported an experiment which they interpreted as demonstrating that the processes of maintenance are of a lower order than those of growth, and that repair of tissue waste can be effected without the amino-acid lysin, whereas this complex is indispensable for growth. There were two confusing factors in this experiment, *viz.*, the "protein-free milk" nitrogen, and the erroneous belief that the protein gliadin contained but little lysin. They restricted a female rat for 178 days to a diet containing gliadin and "protein-free milk." It became pregnant and produced four young. It suckled these to the age of 23 days. This was presented as evidence that maintenance of the mother, the development of young in the uterus, and the formation of milk to nourish the young, are possible on a diet lacking lysin and incapable of supporting growth. Later studies on the content of lysin in gliadin support the view that no differentiation can be made of the nutritive needs of an animal during growth as contrasted with an adult drawing sustenance for the performance of the functions of reproduction and the rearing of young. This mother was simply being nourished on the lowest intake of lysin possible for her requirements under the experimental conditions. Hart, Nelson and Pitz (1918) found with rats that the mammary gland has not the capacity to synthesize lysin, and that this substance is not dispensable for maintenance.

Osborne had carefully determined the amounts of lysin in numerous proteins and had found the content of this substance in gliadin to be 0.16 per cent. This amount is very small compared with the yields obtainable from many other proteins, notably those of animal tissues. This is illustrated by the following table (Osborne and Mendel, 1914):

LYSIN IN PROTEINS

	per cent		*per cent*
Lactalbumin	8.10	Glutelin (maize)	2.93
Halibut muscle	7.45	Glutenin (wheat)	1.92
Ox muscle	7.59	Edestin (hemp seed)	1.65
Casein (cow's milk)	7.61	Amandin (almond)	0.72
Vitellin (egg milk)	4.81	Gliadin (wheat)	0.16
Albumin (hen's egg)	3.76	Hordein (barley)	0.00
Legumin (pea)	4.98	Zein (maize)	0.00
Phaseolin (kidney bean)	4.58		

In another experiment they restricted young rats to a diet consisting of gliadin 18.0, "protein-free milk" 28.2, starch 20.8, agar-agar 5.0 and lard 28.0 per cent. The animals were able to maintain their weight for 500 days, but were unable to grow to any appreciable extent. When lysin was added to the diet to the extent of 3 per cent of the protein content, the animals were capable of normal development.

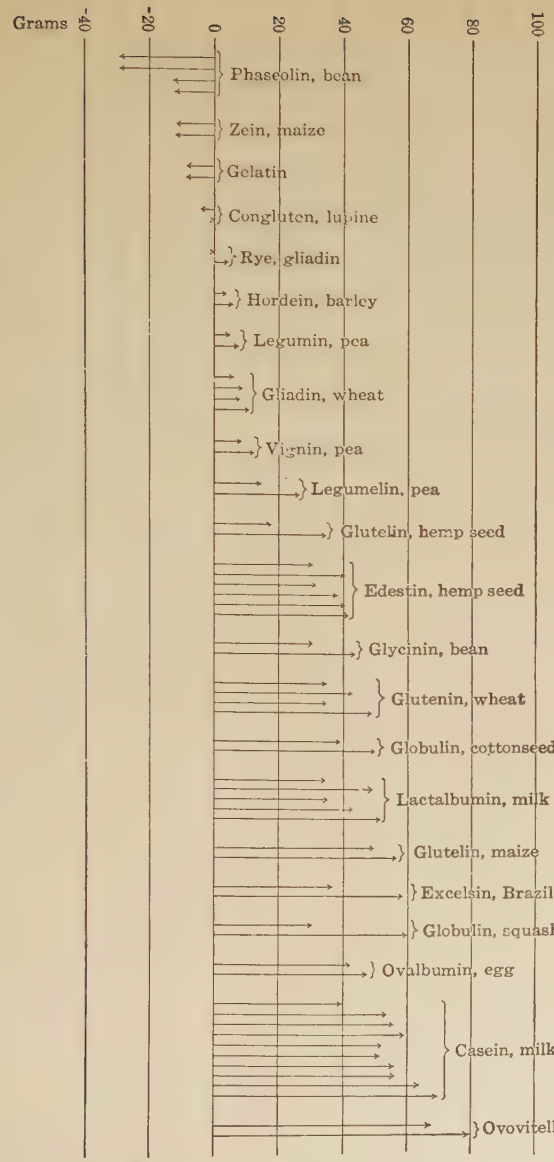
At the time these experiments were conducted with gliadin it was assumed that this protein contained but 0.16 per cent of lysin. Osborne, Van Slyke, Leavenworth and Vinograd (1915) made new analyses of gliadin and other proteins, by a more satisfactory method than that which Osborne had earlier employed (1909). They found that gliadin contains about 1.21 per cent of lysin, or about seven times what it was earlier assumed to contain. With this knowledge available, and with the fact made evident that the uncharacterized nitrogen of "protein-free milk" in the quantities used in their experiments amounts to about half the maintenance requirement of a young animal, there is no reason to regard these data as having any bearing on the problem of the possible dissimilarity in the amino-acid requirements for the processes of maintenance as contrasted with those of growth.

Methods for Comparing Nutritive Values of Proteins.—One method which Osborne and Mendel employed to illustrate the striking differences in the values of the proteins from different sources for the support of growth, was that of feeding a series of groups of animals on a diet of constant composition, but with an isolated and purified protein, as a component in the diet of each experimental group. The amount of growth or of loss in weight of the animals during the first 30 days on the experimental diets was recorded and made the basis of comparison. The diet contained the following substances:

	per cent
Purified protein	18.0
"Protein-free milk"	28.2
Starch	20.8
Agar-agar	5.0
Fat	28.0

The results obtained with a series of proteins are shown in graphic form in the diagram on page 67.

An inspection of this table shows that animals restricted to bean protein lost weight rapidly. Those fed zein and those fed



The above table is slightly modified from a publication by Osborne and Mendel, Zeitschr. f. physiol. Chem., 1912, lxxx, 307.

gelatin lost weight, but not so rapidly. Conglutin, from lupine, and gliadin, from rye, served to maintain weight without growth, and little growth was secured with hordein of barley, or legumin of pea. All the other proteins listed were capable of promoting growth when fed with the other substances in the diet.

A second method which they employed for the comparison of the biological values of different proteins for growth in the rat, was to observe the rapidity of growth of the animals when fed diets of the type described above, in which but a single protein was added. The character of the protein varied in different experiments (1914).

They fed young rats diets, the protein content of which was below the amount which would suffice for the support of growth. The animals remained stunted for long periods. When the amount of protein in their diets was increased they were able to resume growth at rates which Osborne and Mendel regarded as faster than rats ordinarily grow on a "normal" diet. From these results the deduction was drawn that interruption of growth does not result in the loss of the capacity to grow (1914 and 1915).

This generalization is too sweeping. It is true that rats which are stunted by means of protein starvation retain for long periods their capacity to resume growth. The form of the grown animal is closely similar, if not identical, to that of animals whose growth to adult size has been uninterrupted. McCollum and Simmonds (unpublished data) have found that young rats kept upon diets, the inorganic content of which was unsatisfactory, develop abnormal forms, and become deformed. Any nutritive regimen which will induce rickets will markedly interfere with subsequent growth.

Animals whose growth has been suspended by depriving them of vitamins A and B, are able to resume growth upon the inclusion of the missing substance in the diet. It is not possible, however, to carry animals on such diets for long periods, because of the disaster which inevitably befalls them.

A third method was to carefully establish the amount of increase in body weight which was possible as a result of the consumption of a definite amount of protein. The rest of the food mixture remained essentially constant throughout the series of experiments (1915). As an illustration of the results secured by the latter method the following table is representative:

TABLE I

Rat	Diet	Initial body weight	Gain		Intake of	
					Total food	Protein
		grams	grams	days	grams	grams
2115	9% lactalbumin	65	99	80	589	43.7
2123	" "	43	89	80	395	29.3
2207	" "	59	91	80	575	42.6
2210	" "	60	93	80	560	41.5
2051	9% casein (cow's milk)	42	59	80	435	35.2
2110	9% edestin (hemp seed)	52	60	80	465	38.9
2044	4½% lactalbumin (milk)	42	44	80	371	13.8
2049	4½% lactalbumin	41	54	80	454	16.8
2118	4½% casein	59	−3	80	366	14.8
2113	4½% edestin	93	14	80	459	19.2
2114	4½% casein	65	29	80	464	19.4

It will be observed that rats which ate similar amounts of food grew much faster on 9 per cent of lactalbumin than on casein or edestin. Similar differences were observed on the lower protein intake. At the time these experiments were carried out it was believed by Osborne and Mendel that they actually represented quantitative comparisons of the values of these proteins for growth.

Uncertainty Concerning Biological Value of Lactalbumin.—The more recent experimental studies of lactalbumin make it impossible to interpret the results obtained with this protein. Osborne and Mendel (1917) in earlier studies secured growth with lactalbumin when the diet contained "protein-free milk" as a source of the vitamin B, but little or no growth when yeast furnished the vitamin. McCollum, Simmonds and Parsons (1919) were unable to secure growth with a diet in which lactalbumin was the only source of protein.

Sure (1920) also stated "lactalbumin when fed at 12 and 18 per cent of a ration, carrying 2 per cent of the total protein in the form of nitrogen of unknown source in an alcoholic extract of wheat embryo to furnish the water-soluble vitamin, was found to be inadequate for growth. When fortified with a weight of cystin equal to 1 per cent of that of the total protein, lactalbumin fed at 12 to 18 per cent level was found to be of excellent nutritive value. Cystin, then, is the primary limiting factor in that protein." However, Osborne and Mendel have more re-

cently (1924) reported good growth with diets containing only lactalbumin as a source of protein, with a yeast fraction fed as the source of vitamin B. They attribute the different results of the several investigators to a difference in the amount of vitamin B in the diets employed, but state that they are at a loss to explain the failure of the rats to grow better on lactalbumin, edestin, and cotton-seed globulin in the experiments under criticism (1917).

Amino-acid Requirements in Maintenance and in Growth.—Soon after the studies which led Osborne and Mendel (1912) to conclude that the amino-acid lysin is not necessary for maintenance but is indispensable for growth, Osborne and co-workers (1915) made an analysis of the principal proteins of the endosperm of wheat, maize and rice kernels. They found them to yield 1.58, 0.97 and 4.26 per cent, respectively, of lysin. They correlated these results with those which Thomas (1909) secured in brief experiments upon himself and stated: "It is rather striking that the figures for the utilizability of these proteins correspond so closely with the lysin which they yield." The three-day type of digestion trial employed by Thomas, in which the factor of growth is not concerned, can not be regarded as affording data comparable with the standards required in present-day experimental work.

Nollau (1915) and Buckner, Nollau and Kastle (1916) interpreted Osborne and Mendel's data as showing that "lysin is primarily responsible for the stimulation of growth." They apparently regarded the planning of farm rations to insure a sufficient amount of this amino-acid as an agricultural problem of practical importance. They fed chicks such complex mixtures as wheat, wheat bran, sunflower seed, hemp seed and skim milk, and contrasted the growth and well-being of these chicks with others restricted to barley, rice, hominy, oats and gluten flour. The former were well nourished and grew in a normal manner, while the latter were stunted and miserable looking creatures. Buckner, Nollau and Kastle interpreted their results as being due to "differences in the amino-acid content of the two rations and in all probability to differences in the lysin content."

It will be observed that the poor ration was limited to cereal grains and their by-products. One of the most important generalizations that can be made as the result of modern nutrition investigations is that it is not possible to secure a diet com-

posed entirely of seeds which will promote good nutrition over any considerable period. The diet in question was poor in lysin, but its mineral content was deficient in certain elements, notably calcium, phosphorus, sodium and chlorin. Growth was not possible in their chicks, even if the proteins had been of better quality. Furthermore, this diet was very deficient in vitamin A, without which well-being is not possible. The good diet on the other hand was composed in great part of whole seeds, the germ of which improved the quality of the entire mixture of vegetable components to an important degree. The bran was important as a source of mineral salts. When in addition to these differences between the two diets, the presence of skim milk in the successful one is considered, it becames apparent that the two diets were in no sense comparable in regard to factors other than lysin. Without doubt a liberal addition of lysin to their poor diet, or a liberal addition of all the essential digestion products of protein in the form of a purified protein such as casein, would not have changed the outcome of their experiment.

In a later study Osborne and Mendel (1916) employed maize gluten, which contains about 1.0 per cent of lysin, supplemented with "protein-free milk" sufficient to furnish 1.33 per cent of the total nitrogen of the food mixture, as the source of protein for growing chicks. They compared the growth obtained with this protein mixture with that obtained with rations similar in their make-up except that the maize-gluten nitrogen and "protein-free milk" nitrogen was supplemented with lactalbumin or cotton-seed flour. The latter supplements are known to be rich in lysin. The results were interpreted as confirming the conclusions of Buckner, Nollau and Kastle in respect to the effect of foods high and low in lysin on the growth of chicks.

From the standpoint of practical nutrition these experimental studies all tended to focus attention upon lysin as an amino-acid of more importance than other indispensable digestion products of proteins, because it is relatively less abundant in proteins of the cereal grains.

The view that the content of lysin is especially likely to be low in vegetable proteins is reflected in the publications of Johns and his co-workers (1917, 1918), who have emphasized the importance of discovering among the vegetable foods sources of proteins rich in the basic amino-acids, arginin, histidin, and lysin, and especially the latter. This view is justified by the known

peculiarities of the cereal proteins which form so important a source of protein in the nutrition of man and animal. The studies of Osborne and Mendel have shown that the cereal proteins are especially deficient in this group of protein-building units.

No generalization can be made regarding the cause of low biological value of individual proteins. While lysin is the limiting factor in some proteins it appears not to be more frequently so than certain other amino-acids. While it is logical on theoretical grounds to combine a food which is known from the results of chemical studies to be low in lysin, or any other essential animo-acid, with another food especially rich in such compound, it is not safe to place much confidence in the results of chemical analysis in this field of inquiry. This is well illustrated by the deductions regarding the special importance of lysin in foods. McCollum, Simmonds and Pitz (1916) have shown that poor as are the proteins of the maize kernel in lysin, this is not the amino-acid present in smallest amount in proportion to the amount required for the formation of new tissues during growth. It is not the limiting factor in determining the value of the maize kernel proteins. It is not out of place to describe the procedure which established these facts. A food mixture was used which consisted of a single grain so supplemented with inorganic salts and butter fat as to be satisfactory for the promotion of growth, except that the protein was too low in amount to promote growth at the normal rate. In all cases the diets were shown to be made efficient for growth by the addition of a purified protein alone, when that protein was casein of milk. This is a complete protein for the nutrition of an animal. Rations containing wheat, maize or oat kernel were fed to young rats, and the same rations supplemented with gelatin or zein were fed to other groups. Zein, the most abundant protein in maize, is entirely lacking in tryptophan and lysin, whereas gelatin contains about 6 per cent of lysin.

Obviously, if lysin was the limiting amino-acid in each of these three grains, the addition of gelatin should enhance the value of the proteins in all cases and the nutrition of the animals should be correspondingly improved. The addition of zein, on the other hand, should not lead to improvement. The conclusion was drawn from these experiments that zein did not supplement the proteins of the wheat or maize kernels, but did improve the proteins of the oat kernel. Later investigations

FIG. 5.—The rations of these two rats had the same composition as shown by chemical analysis. They differed only in the source of the protein which they contained. The rat on the right grew up on a mixture of proteins from the corn kernel and wheat gluten; that on the left on a mixture of corn proteins and gelatin. The difference in size, and remarkable difference in appearance is solely the result of the difference in the quality of the proteins in the two diets. Corn proteins and gelatin do not supplement each other's amino-acid deficiencies. These animals were the same age when photographed, and had been confined for the same number of days to the experimental diets.

have caused McCollum and Simmonds to question these conclusions so far as the oat kernel is concerned (unpublished data). Lysin may possibly be the limiting factor in the oat kernel. Hogan (1916) found that the addition of lysin and tryptophan to maize did not enhance the nutritive value for growth of swine.

Gelatin supplements the proteins of both the wheat and oat kernel in a very efficient manner, but does not enhance the value of maize kernel proteins. The improvement of diets containing maize gluten, which Osborne and Mendel (1917) observed to follow the introduction of lactalbumin or cotton-seed proteins, was apparently not the result of the addition of lysin.

Some further deductions are possible from these data. They support the view, but do not establish it, that lysin is the limiting factor in oat and wheat proteins. Gelatin contains no tryptophan or tyrosin and but a trace of cystin. This suggests that none of these three amino-acids are the ones present in minimal amounts in wheat or oat proteins. (See Fig. 5.)

Basis of Nutritive Value of Proteins.—The work reviewed clearly establishes that the nutritive value of proteins is determined by their yields of the eighteen or more amino-acids which are formed on digestion. The more nearly these proportions correspond to the content of amino-acids in the tissues of the growing animal, the more effectively can food proteins be transformed into body proteins.

There is no convincing evidence that the processes of maintenance, or the repair of tissue waste, are of a lower order than those of growth, and that the needs of the former are met by a list of amino-acids which would be lacking in certain ones which are essential for the latter. Neither has it been possible to demonstrate that through the agency of the mammary gland, amino-acids (*e.g.*, lysin) can be synthesized for the nutrition of the young. The evidence all points to the conclusion that the transformation of food protein into milk protein is governed by the same laws as apply to growth.

Protein constitutes but a single factor of importance in nutrition. The content of the essential mineral elements in the diet, and of the several unidentified factors, the absence of which leads promptly in each case to the development of pathological conditions, are of equal importance from the standpoint of the well-being of the body. The discovery of the means of making the nice adjustments in a quantitative way among all the factors

best adapted to promote optimum development, and of the means of preserving the harmonious rhythm of the metabolic processes in the best possible manner, is the end sought by students of nutrition. The various factors mentioned will receive attention in later chapters.

CHAPTER V

SOME PROBLEMS OF PROTEIN NUTRITION

Protein as a Structural Material.—The most prominent component of the body tissues with the exception of the skeleton is protein. It is the basis of the muscles and glands, and overshadows all other organic compounds in relative abundance. Liebig believed that a muscle does mechanical work at the expense of energy stored in it in the form of protein. He believed that the energy came from the breakdown of the complex protein into simpler products analogous to the change of complex substances of wood into the products of combustion. He believed that the energy set free was capable of appearing in part in the form of work.

If this view was correct, the amount of protein broken down daily should be profoundly influenced by the amount of work performed. Modern investigations have established, however, that work is ordinarily performed at the expense of the energy of carbohydrates such as starch and sugars, or from fat. This was first shown by Pettenkofer and Voit in 1866. These investigators observed that a fasting man showed no increased protein destruction as measured by the amount of nitrogen eliminated in the form of waste end-products in the urine. Instead of prodigally wasting his muscle substance (protein) he burned fat as a source of energy. Scott and Hastings (1920) have shown recently that *following severe work* there is an increased elimination of nitrogen.

For many years physiologists assumed that the tissues of the human or animal organism were not capable of synthetic transformations so as to give rise to the formation of complex organic compounds from simple ones. The building up of organic substances from inorganic compounds was believed to occur only in plants during growth. The logical inference was that the wear quota of the protein-rich tissues must be replaced by new proteins from the food. Early observations on fasting men and animals established the fact that even during periods of ab-

stinence from food, tissue waste still went on, and in the absence of protein in the diet the muscles and other tissues were used up. During a subsequent period of feeding the lost muscle substance may be completely restored. One of the problems which occurred in the inquiring mind of Carl Voit (1872) was to determine the amount of protein in the form of meat which would suffice to prevent a nitrogen (protein) deficit in dogs. He did not actually determine the minimum amount of protein necessary to accomplish this. He showed that with moderately large intakes of meat protein, the nitrogen which appeared in the urine as waste products of metabolism, plus that eliminated in the feces, was equal to that ingested in the food. In other words the body was in nitrogen (or protein) equilibrium.

Gelatin an Incomplete Protein.—Gelatin, which results from incipient hydrolysis of connective tissue and the organic matrix of bone, is a protein of peculiar nature. It was one of the first to be studied by chemists. Gelatin and the casein of milk were among the first proteins to be separated in a state of approximate purity. Voit attempted to substitute gelatin for a part or all of the meat protein in the diets of his dogs and discovered that gelatin as the sole source of nitrogen could not prevent loss of body protein. There was something lacking in its makeup which made it an incomplete food. Gelatin became therefore a substance of unique interest to physiological chemists.

Millon (1849) discovered a reagent which gives a characteristic brick red color when added to most proteins. Gelatin does not exhibit this property. Nasse (1879) demonstrated that the reaction of Millon is due to the presence in the protein molecule of the amino-acid, tyrosin. This is one of the digestion products of many proteins. Gelatin does not yield tyrosin when digested. It has still other deficiencies which were later discovered. Tryptophan and the sulphur-containing amino-acid, cystin, are both lacking in its molecule. Gelatin has long been known to be an "abnormal" or incomplete protein, and its value in nutrition has been the subject of many investigations.

Örum (1879) and Munk (1894) confirmed the earlier observations that a part of the protein could be substituted by gelatin, but that when it forms the sole source of nitrogen there is always a deficit.

Kossel discovered the chemical nature of the protein molecule to be a chainlike structure consisting of a number of amino-

acids linked together. The amino-group of one is united to the carboxyl group of its neighbor in the chain through abstraction of the elements of water. Digestion consists of the addition of the elements of water and the dissolution of the union. The correctness of this view was proven by Curtius and by E. Fischer, through synthesis, under the guidance of Kossel's theory. This is discussed in Plimmer's monograph on the Chemical Constitution of the Proteins (1912).

Objectives of Research on Protein Nutrition.—Researches in the field of protein nutrition during the last 25 years have had as their main objectives the solution of the following problems: (1) The digestibility of the proteins; (2) the number and nature of the amino-acids contained in proteins of plant and of animal origin; (3) the amounts of each of these yielded by different proteins; (4) the extent of the digestive decomposition of proteins in the alimentary tract; (5) the ability or inability of the tissues to synthesize the various digestion products of protein; (6) the immediate disposition of the products of protein digestion after absorption; (7) the chemical changes which take place during the catabolism or destruction of the amino-acids in the body; (8) the capacity of the body to synthesize protein when the requisite amino-acids are supplied in the food; (9) the determination of the relative efficiency possible in the transformation for purposes of growth of (a) individual proteins and of (b) the mixture of proteins in individual foodstuffs into body proteins; (10) a comparison of the number and kinds of amino-acids required in the process of repair of tissue waste, which is an essential result of life activities, with the requirements for the formation of new tissues during growth; (11) the physiological effects of nutrition with protein intake at different planes, or the discovery of the amount of protein from stated sources which serves to maintain the body in optimal nutritive condition.

It is not within the scope of this book to discuss all phases of this subject. An arbitrary selection of topics has been made, and only the last four problems will be considered. This is desirable because the literature relating to research along these lines is especially in need of being summarized and interpreted.

After the composition of the proteins became known, physiological chemists interested themselves in trying to demonstrate which, if any, of the 18 or more amino-acids or digestion products of proteins are indispensable in the diet. Henrique and Hansen

(1904) published studies which led them to believe that the three diamino-acids, arginin, histidin and lysin, are not necessary in the nutrition of the rat. Their experiment consisted in keeping a nitrogen balance on rats whose diets were believed to be lacking in these amino-acids. They doubtless failed to remove completely from their protein the amino-acids in question.

Kaufmann (1905) conducted the first experiment of a type which has been greatly extended by others. He fed dogs on diets in which gelatin was supplemented with tyrosin, cystin and tryptophan. It was found possible with this mixture to replace fully the protein lost through metabolism. These results he confirmed by experiments on himself. In 1905, when his investigations were made, no one suspected the necessity of including in the diet the three unidentified factors, vitamins A, B and C, which are concerned with the prevention of the development of a type of ophthalmia, polyneuritis and scurvy, respectively. His experiments were apparently so short that these omissions in the diet did not seriously interfere with the outcome of the study.

Researches of Folin on Protein Metabolism.—Folin (1905) published three remarkable papers. He made it clear that the process of the destruction of the greater part of the protein which is digested and absorbed, and of its conversion into the waste products which constitute the normal components of the urine, differs markedly from that type which is involved in the normal functioning of the tissues of the living body. The end-product of the first type is principally urea, while creatinin and uric acid are prominent components of the latter. When a diet free from protein or other nitrogenous compounds is taken in amount sufficient to cover the energy requirements of the body, the urine will, after a few days, contain about 60 per cent of its total nitrogen in the form of urea, 18 per cent as creatinin, 7.0 per cent as ammonia, and 3.4 per cent as uric acid. The remainder, which will amount to about 12-14 per cent of the total, is in forms which are in great measure uncharacterized, and which can not as yet be estimated individually. A urine having this composition represents what Folin termed endogenous metabolism. He distinguished it from exogenous metabolism, which is concerned with the destruction of food protein that has never become a part of the living protoplasm. The magnitude of the endogenous process is surprisingly constant, where-

as that of the latter is very variable, and is determined by the consumption of protein. On ordinary planes of nutrition, exogenous metabolism or destruction of food is superimposed upon the endogenous or tissue waste type and the composition of the urine gives a composite picture of the two.

Willcock and Hopkins' Studies on Zein.—Willcock and Hopkins (1906) made a study of the incomplete protein, zein, which was comparable with Kaufmann's studies with gelatin. Zein, which is the principal protein of maize, is entirely lacking in both lysin and tryptophan. These investigators fed diets of purified food substances to young mice in which zein was the sole source of protein (amino-acids). They observed that the animals died in less than 14 days in a state of torpor. When tryptophan was added to the food they lived about twice as long and remained lively until shortly before death. The addition of tyrosin did not improve the diet. They suggested that tryptophan may serve a two-fold function. Since it is an essential structural unit of the protein molecules they thought it might be a precursor of a substance which certain tissues elaborate, and was of the nature of a hormone or regulator of metabolism, without which death would supervene sooner than through specific starvation for protein alone.

It has been abundantly demonstrated by several workers that the simplest of the amino-acids, glycocoll, is readily synthesized by the animal tissues (Magnus Levy, 1907). Casein contains no glycocoll but is a complete protein, since it can meet all the requirements of an animal for nitrogen in the form of amino-acids. From these results it is safe to conclude that the tissues of the higher animals are capable of synthesizing certain amino-acids. The ones which can be so synthesized are unknown.

Michaud (1909) sought to verify the theory that the more similar the food proteins to those of the tissues which they are destined to form in the body, the higher would be their biological value as nutrients. He allowed dogs to fast for a time to reduce their nitrogen output to the minimum of tissue destruction. Then he fed a non-nitrogenous diet supplemented with various amounts of protein from different sources. His findings indicated that the proteins of the same species of animal are superior in dietary value to proteins obtained from another species or from plants. Dog muscle and dog serum proteins were of higher nutritive worth for the dog than muscle or serum proteins from the horse. A mixture of dog tissues (muscle,

blood and glandular organs) was also superior to any of the other proteins studied. Gliadin and edestin, proteins from wheat and hempseed respectively, were of the lowest value. We know that his diets were not comparable in their quality with respect to factors other than protein. It is now known that all except those in which the glandular organs were included were very deficient in the vitamins, as well as very poor in their mineral content. It is not now possible to accept his interpretation of his results.

Protein Requirements in Maintenance and in Growth.—McCollum (1911) attempted to utilize Folin's discovery as a means of studying the problem whether an amino-acid supply which is inadequate for the support of growth would be satisfactory for maintenance. A farm pig was given a nitrogen-free diet of starch, salts and water until the exogenous metabolism disappeared, as shown by the composition of the urine. In a subsequent period a definite amount of an incomplete protein such as gelatin or zein was added to the experimental diet. From the increase in the nitrogen output in the urine and from the change in the forms in which it appeared, it was believed to be possible to decide to what extent the amino-acids of the incomplete protein were utilized.

It was found that young pigs readily ate a sufficient amount of the nitrogen-free diet to cover their energy requirements, and could easily be brought into a condition in which they exhibited only the endogenous type of metabolism. When a known amount of nitrogen in the form of urea, an end product of metabolism, was added to the diet of pigs in this condition, it was all recovered in the urine *in addition to the amount derived from endogenous sources.* The percentage composition of the urine with respect to creatinin and urea showed the unexpected alterations, the former representing less and the latter more of the total nitrogen of the urine.

When gelatin or zein was administered instead of urea in quantities sufficient to supply exactly the amount of nitrogen derived from endogenous metabolism, *the nitrogen from these "incomplete" proteins was only in part excreted in the urine in addition to the amount derived from endogenous sources.* The results showed that 60 per cent of the nitrogen of gelatin was excreted and 40 per cent retained for some purpose in the tissues, presumably for the partial repair of tissue waste. Under the same experimental conditions 73 per cent of the nitrogen of zein

was retained and 27 per cent excreted in addition to the amount expected from endogenous sources. The same criticism now applies to these experiments as to those of Kaufmann (1905). (See page 80.)

Studies on Utilization of Ammonia Nitrogen by Animals.—Grafe (1912) said that nitrogen equilibrium could be attained on a diet of carbohydrate and ammonium acetate. Abderhalden (1912) repeated this work and found a partial utilization of ammonium salts for replacing the nitrogen lost through tissue waste, but denied the possibility of attaining nitrogen equilibrium. He suggested that the sparing action of ammonium salts is due to prevention of destruction of some of the amino-acids derived from all tissues, and that these may be used over again. Underhill and Goldschmidt (1913) confirmed the view that there is under these conditions a sparing action of body protein by ammonium citrate, but all the tissue loss could not be replaced from nitrogen in the form of ammonium salts. In all these studies we now know that the diets employed were deficient in the vitamins.

The problem concerning the ability of the tissues to take a mixture of amino-acids and combine them into proteins for incorporation into living protoplasm has been studied extensively by Abderhalden (1913). In one of his most successful experiments a dog was fed for 100 days upon meat which had been digested to amino-acids (erepton) by the successive action of pepsin, trypsin and erepsin. These are the protein-digesting enzymes of the stomach, pancreas and intestine, respectively. The digested meat was supplemented with non-nitrogenous nutrients. He reports that the animal remained in good health and gained 9.35 kg. in weight. It was demonstrated by such experiments that protein synthesis is possible when the diet furnishes all the essential amino-acids which serve as structural units in the formation of tissue proteins.

Abderhalden (1913) sought to remove certain amino-acids from completely digested proteins. By means of feeding experiments with the protein digestion mixtures rendered incomplete he sought to determine whether any of the digestion products are indispensable in the diet. The object was to determine whether it was possible for the body to convert one amino-acid into another. He was able to remove most of the tyrosin and tryptophan from his digestion mixtures. The former of these is a cyclic compound and the latter a still more complex

substance containing two types of cyclic structures in its molecule. He showed that these two amino-acids must be furnished in the diet if satisfactory nutrition is to be attained.

McCollum (1914) and Hart and McCollum (1914) sought to compare in growth value the proteins of wheat, maize and oat kernels in young swine. They confined the animals in metabolism cages, thus making possible the collection of the excreta during a period of 30 to 60 days. The animals were fed exclusively on a single grain and water. From the record of the nitrogen intake and output they calculated the amount of protein of the food which was converted into tissue proteins for new growth. The results indicated that the protein mixture contained in each of these grains can be utilized to about the same extent. The grains are deficient in vitamin A, and are almost entirely lacking in vitamin C. Plimmer (1920) has shown that the pig is susceptible to scurvy, and the lack of the latter was perhaps a factor of importance in determining the outcome of the experiments. The lack of vitamins A and C certainly influenced the results of these studies, for the rate of nitrogen retention fell off toward the end of the period of observation.

Body Protein Synthesized from Amino-acids.—Folin and Denis (1913), Van Slyke and Meyer (1913) and Davis and Whipple (1919) have shown beyond a doubt that protein is readily synthesized from amino-acids. In the intestine the protein is digested to the amino-acid stage, and the identity of the food proteins is lost. In the process of growth, in the regeneration of liver tissue destroyed as the result of chloroform narcosis, or in the replacement of the complexes in the tissues lost through the disintegration of certain structures in normal metabolism, the amino-acids derived from food protein are used for the construction of new proteins of structures different from those of the food.

Feeding Experiments with Amino-acids.—Mitchell (1916) conducted experiments with mice fed a diet modeled after one which was extensively employed by Osborne and Mendel. He replaced the protein by a definite mixture of amino-acids. The diet contained "protein-free milk," which furnished approximately half a maintenance ration of protein, but this fact was not known at the time. The efforts to keep mice alive on this diet containing from eight to fifteen amino-acids were unsuccessful. This was in part due to the failure of the animals to eat a sufficient amount of the food to meet their energy re-

quirements. It was found necessary to feed the mice on alternate days on the portion of the food mixture minus the amino-acids to induce them to take a greater amount of food. He found the omission of tryptophan of greater significance than of any of the other amino-acids. Rose and Cos (1924) have recently achieved much greater success in feeding amino-acids instead of protein.

Ackroyd and Hopkins (1916) fed mice the essential non-nitrogenous nutrients supplemented with simple mixtures of purified amino-acids. They concluded that if arginin is present in the diet, histidin may be omitted, and vice-versa. A similar relationship they found between tyrosin and phenylalanin, the structural formulas of which are closely similar. This was the first instance where it was believed that it was demonstrated that one amino-acid may be converted into another.

Hopkins' (1916) data show that tyrosin, tryptophan, and probably all the other cyclic amino-acids and cystin can not be synthesized by the animal.

Geiling (1917) digested casein of milk to the amino-acid stage and then precipitated the arginin, histidin and lysin with phosphotungstic acid. He fed mice the portion of the protein without these diamino-acids. His diets also contained "protein-free milk" which, as has been shown, contained protein nitrogen sufficient to vitiate in great measure the results of his experiments. He interpreted his findings to indicate that arginin and histidin are interconvertible in the body. He also concluded that lysin is not necessary for maintenance without growth.

Chemical Composition of Protein.—It has been mentioned that the studies of Kossel, of Fischer and of Osborne had made it clear that there existed very pronounced differences in the composition of the proteins from different sources. The proteins were prepared in a state of relative purity and were digested in the laboratory by means of acids, and were analyzed by the methods of Fischer and of Kossel. Certain of the eighteen digestion products, the amino-acids, were determined quantitatively so far as the methods would permit. Although the technique was never perfected so as to give results approximately quantitative, except in the case of less than a third of the amino-acids known to be formed in the digestion of proteins, it was shown in the case of these few amino-acids that there were very great variations in the proportions among them in the mixtures obtained from proteins of different sources. Thus the proteins

of the muscle tissues of several species of animals were shown to yield between 12 and 14 per cent of glutamic acid, one of the products of hydrolysis obtained from practically all proteins. This amino-acid is present in the two principal proteins of the wheat kernel to the extent of about 40 per cent of the total protein content. These two proteins together make about 85 per cent of the total protein of the wheat kernel. Other equally great differences were shown to exist in the composition of proteins of our common food-stuffs, and those of the tissue proteins formed during growth.

The problem which the animal meets in its protein nutrition may be illustrated by comparing the digestion products of the protein molecule to the letters of the alphabet. The protein of the food and of the tissues may be regarded as made up of the same letters arranged in different orders and present in different proportions. In growth the animal takes as food proteins which are very unlike those of its tissues. These are taken apart into simple compounds, the amino-acids, which are absorbed and put together in new order, and in new proportions to form the tissue proteins.

If the muscle tissue of an animal be compared to a block of printer's type so arranged as to print the rhyme beginning "Jack Spratt could eat no fat, and his wife could eat no lean," the proteins of which the muscle consists are represented by the individual words, and the protein digestion products by the letters of which the words consist. If now the animal should take food proteins which correspond to a block of type which would print the jingle beginning "Peter Piper picked a peck of pickled peppers," it is easy to understand that when the proteins of the food are resolved into their constituent letters, and an effort is made to form the body proteins of the new and different type from the letters supplied by the food, the transformation can not be made. In setting up the first line, "Jack Spratt could eat no fat and his wife could eat no lean," we need four of the letter t, but the food proteins contain but one. The first line of the Jack Spratt rhyme, which represents the muscle proteins, contains but one letter p, whereas the food proteins expressed by the Peter Piper rhyme yield nine in the first line. The first line of the Jack Spratt rhyme contains the letters j and n, whereas the Peter Piper rhyme contains some, so that even with the entire stanza:

Peter Piper picked a peck of pickled peppers,
If Peter Piper picked a peck of pickled peppers,
Where's the peck of pickled peppers
That Peter Piper picked?

it is not possible to reproduce even the first line of the Jack Spratt rhyme, and in order that growth might become possible it would be necessary to take proteins of another character which would supply the missing letters.

Such a comparison between food proteins and tissue proteins illustrates the kind of problem which the animal meets in its protein nutrition. Zein, of corn, is entirely lacking in three of the animo-acids or digestion products which are obtainable from most tissue proteins. In accord with what we should expect on theoretical grounds, this protein is not capable of supporting growth, or of maintaining an animal in body weight when taken as the sole source of amino-acids. This illustration shows how we may have superior, good or inferior food proteins for the formation of body proteins in growth.

CHAPTER VI

PECULIARITIES OF COMPOSITION OF PROTEINS FROM VARIOUS SOURCES

Certain Amino-acids Indispensable for Nutrition.—In Chapter V an account was given of the experimental studies which established the fact that the nutritive value of a protein depends upon the number and amounts of the amino-acids it yields on artificial hydrolysis or on digestion. It was shown that proteins such as gelatin, lacking in the amino-acids, tyrosin, cystin and tryptophan; or zein of maize, deficient in lysin, are incomplete for all physiological purposes, since they cannot supply all the "building stones" necessary for the construction of body proteins. The nutritive value of a protein or mixture of proteins depends upon the presence in its molecules of all the essential amino-acids, and upon the extent to which their proportions correspond to those existing in the body proteins into which they are to be transformed. One protein may supplement the deficiency of another. On these facts was based a criticism of certain of the experimental work designed to yield data showing in a quantitative way the comparative values of different purified proteins.

In order to illustrate further the differences in the constitution of individual proteins from different sources a few tables are presented which contain in summarized form the best analytical data which it has been possible to obtain by chemical methods. Table II shows the composition of a series of animal and vegetable proteins and the sources of origin. The elements carbon, hydrogen, nitrogen, sulphur and oxygen, are designated by the symbols, C, H, N, S and O respectively.

Elementary Composition of Proteins.—The most interesting information to be gained from this table is the remarkable similarity of the proteins from various sources in respect to their content of carbon, hydrogen and oxygen, and their relatively wide differences in nitrogen and sulphur content. Ovalbumin of the hen's egg contains but 15.51 per cent of nitrogen, whereas

PERCENTAGE COMPOSITIONS						FORMULA						
Source of Protein	*C*	*H*	*N*	*S*	*O*	*Name of Protein*	*C*	*H*	*N*	*S*	*O*	*Molecular weight*
						Plant Proteins						
1. Almond	51.30	6.90	19.32	0.429	22.05	1. Amandin	638	1030	206	2	206	14930
2. Pea	52.64	6.95	17.25	0.426	22.73	2. Vignin	660	1040	185	2	214	15038
3. Bean	51.72	6.95	18.04	0.385	22.90	3. Legumin	718	1158	214	2	238	16642
4. Maize	55.23	7.26	16.13	0.600	20.78	4. Zein	736	1161	184	3	208	15993
5. Soy bean	52.12	6.93	17.53	0.710	22.71	5. Glycinin	780	1248	206	4	255	17700
6. Barley	54.29	6.80	17.21	0.847	20.53	6. Hordein	675	1014	181	4	194	14880
7. Hempseed	51.50	7.02	18.69	0.88	21.91	7. Edestin	624	1021	193	4	199	14523
8. Bynin	55.03	6.67	16.26	0.84	21.20	8. Bynin	706	1026	180	4	204	15410
9. Wheat	52.72	6.86	17.66	1.027	21.73	9. Gliadin	685	1068	196	5	211	15568
10. Para nut	52.18	6.92	18.30	1.086	21.51	10. Excelsin	642	1018	192	5	198	14738
11. Wheat	53.02	6.84	16.80	1.280	22.06	11. Leukosin	663	1026	180	6	207	15006
						Animal Proteins						
12. Ox	54.98	7.20	16.89	0.42	20.51	12 Globin	700	1098	184	2	196	15274
13. Ox	52.68	6.83	16.91	1.10	22.48	13. Fibrin	645	1004	178	5	207	14708
14. Horse	52.71	7.01	15.85	1.11	23.32	14. Serumglobulin	628	1002	160	5	209	14310
15. Horse	52.93	6.90	16.66	1.25	22.26	15. Fibrinogen	679	1062	183	6	207	15276
16. Muscle	52.82	7.11	16.67	1.27	22.03	16. Myosin	660	1074	181	6	208	15048
17. Egg	52.75	7.10	15.51	1.616	23.02	17. Ovalbumin	696	1125	175	8	220	15703
18. Cow's milk	52.19	7.18	15.77	1.73	23.13	18. Lactalbumin	644	1064	166	8	214	14792
19. Horse	52.99	7.01	15.93	1.93	22.14	19. Serumalbumin	662	1051	171	9	207	14989
20. Man	52.25	6.65	15.88	2.25	22.95	20. Serumalbumin	684	1045	178	11	225	15697
21. Horse	54.64	7.09	17.38	0.39	20.16	21. Oxyhaemoglobin	758	1181	207	2	210	16655
22. Man	54.57	7.11	16.38	0.568	21.04	22. Oxyhaemoglobin	758	1185	195	3	219	16667
23. Cow's milk	53.13	7.06	15.78	0.800	22.37	23. Casein	708	1130	180	4	224	15982
24. Egg yolk	51.56	7.12	16.23	1.028	23.24	24. Ovovitellin	671	1112	182	5	227	15628

* Osborne, T. B. Zeit. f. analyt. Chem., xli, 25, 1902.

amandin from the almond contains 19.32 per cent. Legumin from the bean contains but 0.385 per cent of sulphur, while serumalbumin from human blood contains 2.25 per cent of this element, or nearly six times as much for equal weights of the two substances.

The Hausmann Method of Analysis of Proteins and the Data Obtained.—Table III shows data regarding the constitution of different proteins as revealed by the Hausmann method of analysis. There is in every protein a complex which yields ammonia when boiled for a few hours with moderately strong mineral acids. In the table the per cent of the total nitrogen of the protein which is convertible into ammonia is tabulated in the first column under NH_3-N. There are three diamino-acids derived from proteins. These are sharply differentiated from all the others by their basic character, that is, they have an alkaline reaction in solution. They can be precipitated together by means of phosphotungstic acid, and thus separated sharply from all the other constituents of the protein molecule. The remaining amino-acids formed on hydrolysis or digestion of proteins are collectively designated as mono-amino acids. When proteins are heated with acids as is necessary in the method of analysis under discussion, there is always formed a certain amount of black, insoluble nitrogenous substance called humin. It has been shown that most of this comes from the decomposition of a single compound called tryptophan, one of the mono-amino acids. Some proteins (*e.g.*, zein) are entirely lacking in tryptophan. Under "Total N," is given the per cent of nitrogen in the samples employed in the analyses.

An inspection of this table shows that the amount of nitrogen in the protein molecule which is convertible into ammonia under the conditions of the analysis varies from 6.46 per cent in legumelin of the pea or bean to 23.78 per cent in gliadin of wheat. In contrast with the proteins of animal origin, it will be noticed that many of the proteins of vegetable origin contain two to four times the amount of nitrogen in this special ammonia-yielding complex. Data of this kind as early as 1905 clearly showed that the proteins differ to a great extent in their constitution, and that food proteins are in most cases quite unlike the proteins which compose the muscles and organs of the body.

The method of analysis by means of which the data in Table III were secured is not a very searching one, and fails to yield

TABLE III

Distribution of Nitrogen in Proteins*

TOTAL N = 100

	NH_3 N	*Basic Amino Acids* N	*Mono-Amino Acids* N	*Humin* N	*Total* N
Vegetable Proteins:					
Conalbumin (egg white)	7.51	25.82	65.11	1.61	16.11
Phaseolin (bean)	10.74	24.50	62.85	1.79	16.20
Glycinin (pea).....	12.09	22.63	64.58	.68	17.45
Legumelin (pea, bean)..	6.46	23.06	68.12	2.36	16.09
Leucosin (wheat)	6.85	20.67	69.87	2.54	16.93
Glutenin (wheat).....	18.86	11.72	68.32	1.09	17.49
Gliadin (rye)	23.78	5.49	70.27	.79	17.66
Hordein (barley)	23.30	4.47	69.96	1.33	17.21
Zein (maize)	18.41	3.03	77.56	.99	16.13
Globulin (wheat)	7.72	37.14	53.39	1.52	18.39
Globulin (coca nut)....	7.35	32.79	59.09	.75	18.48
Globulin (squash seed)..	6.91	32.25	59.64	1.18	18.51
Globulin (cotton seed)..	10.30	30.63	59.06	....	18.64
Edestin (hemp seed)....	10.08	31.70	57.83	.64	18.64
Excelsin (para nut)....	8.87	31.47	59.94	.92	19.30
Corylin (hazel nut).....	11.57	30.26	56.31	.89	19.00
Amandin (almond).....	16.04	21.84	60.08	.89	19.00
Globulin (castor bean)..	10.45	30.08	58.66	.64	18.75
Corylin (walnut)	9.44	28.71	61.09	.79	18.84
Congluten (a) (lupine)..	11.84	29.05	58.00	1.00	17.90
Congluten (b) (lupine)..	14.55	28.17	56.56	.76	18.21
Legumin (pea)	9.40	28.82	60.27	.94	17.97
Globulin (flaxseed)	10.82	25.81	62.06	1.18	18.48
Vicilin (pea, bean).....	10.40	27.76	60.60	1.22	17.11
Vignin (pea)	11.07	25.39	62.66	1.45	17.25
Globulin (sunflower seed)	13.83	22.98	62.00	1.29	18.58
Stizolobin (Velvet bean)	10.51	27.96	60.06	1.36	16.40
Arachin (Peanut)	11.10	27.13	60.50	1.20	18.28
Animal Proteins:					
Vitellin (egg yolk).....	7.67	28.56	62.41	1.35	16.28
Casein (cow's milk).....	10.30	22.84	66.00	1.34	15.62
Ovalbumin (egg).....	8.64	21.27	68.21	1.87	15.51
Chicken muscle	7.45	29.98	59.85	2.43	16.09
Halibut muscle	6.70	30.18	60.73	2.37	16.40
Scallop muscle	6.33	26.51	64.81	2.34	17.05
Beef muscle	5.50	27.32	64.52	2.65	16.18

* Compiled from the papers of T. B. Osborne and of C. O. Johns.

as detailed information as is desired for physiological studies. For this purpose the differences in the yields of the diamino-acids (bases) in different proteins do not, except in a few instances, contrast markedly with each other. In the case of glutenin, gliadin, hordein and zein, the principal proteins of the wheat, barley, and maize kernels, the content of the basic amino-acids falls below that present in any of the animal proteins. As will be seen from Table IV, there is more to be learned about this fraction of the protein molecule than the above data reveal. There are three amino-acids in this group, called arginin, histidin and lysin. Different proteins show marked variation in the content of one or another of these.

The Content of Arginin, Histidin and Lysin in Individual Proteins.—Table IV contains analytical data obtained by the Kossel method for the estimation in proteins of arginin, histidin and lysin. The histidin content of plant proteins varies much less than does that of either arginin or lysin. The extreme values for this constituent of the protein molecule range from 3.81 per cent in stizolobin from the velvet bean, to 0.39 per cent in gliadin from rye. The content of arginin in different proteins varies in a surprising degree. A globulin of buckwheat yields 23 per cent, whereas zein contains but 1.35 per cent. Among the vegetable proteins the yield of lysin is generally very low in comparison to that obtained from the proteins of animal tissues. The extreme limits of the yield of lysin in plant proteins range from 10.2 per cent in the globulin of the velvet bean to complete absence in zein.

A comparison of the relative amounts of these three digestion products in vegetable proteins with those of animal origin reveals at once a remarkable fact. While the amount of histidin is of the same order in the two classes, arginin is found in many proteins of seeds in quantities greatly in excess of any of the proteins of animal tissues. The only vegetable proteins yet studied which exceed the muscle proteins in their content of lysin are those of the velvet bean and buckwheat (Johns and Co-workers 1918, 1920).

It was the marked deficiency of most of the vegetable proteins in lysin in contrast to the animal proteins that led Osborne and Mendel and others to believe at one time that this amino-acid was frequently the limiting factor which determined the nutritive value of food proteins.

Variation in Glutamic Acid Content of Proteins.—Table

TABLE IV

CONTENT OF ARGININ, HISTIDIN AND LYSIN IN PROTEINS.*

	Histidin	*Arginin*	*Lysin*
Vegetable Proteins:			
Globulin (Buckwheat)	0.88	23.67	8.61
Globulin (Squash seed)	2.42	14.44	1.99
Excelsin (Para nut)	2.50	14.20	1.64
Edestin (Hemp seed)	2.19	14.17	1.65
Globulin (Cotton seed)	3.46	13.51	2.06
Stizolobin (Velvet bean)	3.81	14.13	10.02
Globulin (Castor bean)	2.74	13.19	1.54
Amandin (Almond)	1.87	12.16	0.72
Legumin (Pea)	1.69	11.73	4.98
Legumin (Vetch)	2.94	11.06	3.70
Conglutin A (Lupine)	2.51	10.93	2.73
Vicilin (Pea)	2.17	8.91	5.40
Glycinin (Soy bean)	2.10	7.69	3.39
Vignin (Cow pea)	3.08	7.20	4.31
Glutelin (Maize)	3.00	7.06	2.93
Leucosin (Wheat)	2.83	5.94	2.75
Legumelin (Pea)	2.27	5.45	3.03
Legumelin (Soy bean)	2.04	5.35	4.91
Phaseolin (Kidney bean)	2.62	4.87	4.58
Glutenin (Wheat)	1.76	4.72	1.92
Gliadin (Wheat)	0.58	3.16	1.33
Gliadin (Rye)	0.39	2.22	...
Hordein (Barley)	1.28	2.16	...
Zein (Maize)	0.82	1.35	0.00
Animal Proteins:			
Ovovitellin (Egg yolk)	1.90	7.46	4.81
Beef muscle	2.66	7.47	7.59
Scallop adductor muscle	2.02	7.38	5.77
Chicken muscle	2.47	6.50	7.24
Halibut muscle	2.55	6.34	7.45
Conalbumin (Egg white)	2.17	5.07	6.43
Ovalbumin (Egg white)	1.71	4.91	3.76
Casein (Cow milk)	2.50	3.81	5.95
Lactalbumin	2.06	3.23	9.16

* This table is compiled from various sources, especially the papers of Osborne and of Johns.

V shows the composition of a number of proteins of animal and of vegetable origin with respect to glutamic acid. This amino-acid is a never-failing component of all vegetable proteins and of all animal proteins except the protamins in the spermatozoa of fish. It will be seen that this hydrolytic product is frequently

much more abundant in vegetable proteins than in animal tissues or in egg or milk proteins.

TABLE V

PER CENT OF GLUTAMIC ACID YIELDED BY PROTEINS *

Vegetable Proteins:	
Gliadin (Wheat)	42.98 per cent.
Gliadin (Rye)	37.80
Hordein (Barley)	43.19
Zein (Maize)	26.17
Leucosin (Wheat)	5.72
Glutenin (Wheat)	23.40
Amandin (Almond)	23.14
Edestin (Hemp seed)	14.00
Corylin (Hazel nut)	17.94
Phaseolin (Bean)	12.33
Vignin (Pea, bean)	16.30
Legumin (Vetch)	16.48
Glycinin (Soy bean)	19.46
Conglutin (Yellow lupine)	30.05
Animal Proteins:	
Casein (Cow milk)	10.77
Ovalbumin (Egg)	9.01
Conalbumin (Egg)	7.00
Vitellin (Egg yolk)	12.95
Serum albumin (Horse)	7.70
Serum globulin (Horse)	8.50
Halibut muscle	10.13
Chicken muscle	16.48
Scallop muscle	14.88
Dog tissues, entire body	14.00
Rabbit tissues, entire body	14.14
Hen tissues, entire body	12.02

* Compiled data from various sources, especially the papers of T. B. Osborne and E. Abderhalden.

With these data available, it is easy to understand why food proteins show great variations in nutritive value, for this depends upon the efficiency with which they can be transformed during growth, or in the course of the repair of tissue waste into tissue proteins. Since in human and in animal nutrition several proteins are always taken together, when vegetable or animal tissues are used as food, it is of great economic importance to discover what foods contain proteins which possess high supplementary values. Protein is the most expensive component of foods or feeding-stuffs, and agricultural economics can be greatly

advanced by scientific discovery in this field. In human nutrition the element of economy, in ordinary times, enters less as a factor than physiological well-being, but it would appear that it is no less important here that a protein supply of high biological value be provided in the diet. It seems logical to prefer to serve the needs of the body by providing it with the optimal amount of such proteins as can be utilized very effectively rather than to require it to digest and assimilate an excessive amount of proteins of low value. In this connection it must be remembered that the moiety of animo-acdis which cannot be properly matched with each other so as to form the necessary structures have to be destroyed and their degradation products eliminated as waste. This imposes an unnecessary burden on the organs concerned with the metabolism of the nitrogenous components of food.

CHAPTER VII

SUPPLEMENTARY VALUES OF PROTEINS

Methods for the Study of Protein Values.—Since chemical methods for the analysis of proteins have not been perfected so as to give a satisfactory knowledge of the differences in their composition, it is necessary to study this problem by means of experiments on animals. Several lines of procedure offer prospects of successful comparison of the relative biological values of mixtures of proteins. In order to study the effectiveness with which the proteins of one food supplement those of another it is essential that diets be planned so as to be highly satisfactory with respect to all factors other than protein. The natures of the deficiencies of all the foods studied must first be well understood, so that any mixtures employed in experiments designed to reveal the biological values of combinations of proteins may be supplemented with respect to inorganic salts, the essential vitamins, etc. Under such considerations the success or failure of growth, reproduction or other function in the animals, will turn solely on the quality of the proteins of the experimental diet. In Chapter IX the dietary properties of many of the more important of our foods are given in detail. It is now appropriate to present the method employed in the study of the protein values of combinations of these articles of diet. One procedure is to feed a series of groups of young animals a diet similarly constituted and deriving all of its protein from the same source, but with the per cent of protein adjusted in the series from low to high levels. From the rate of growth of the animals an idea can be gained as to the least amount of protein from the sources studied which just suffices to maintain animals without growth and to induce growth at subnormal rates, or at the maximum rate.

Another method of investigation is to utilize female animals nursing litters of young. By adopting a standard size for the litter, and by observing the success with which the mothers induce growth in their young when the diet contains different percentages of protein but is otherwise of uniform composition,

an approximately quantitative comparison of the values of the proteins from different sources can be made.

Osborne and Mendel (1917) made attempts, in their studies with isolated proteins, to determine their relative values by observing the growth of animals which ate the same amount of food in the same number of days and gained the same amount in weight. The protein factor was the only variable. It is difficult to conduct experiments in this manner, and Osborne and Mendel (1919) attempted to develop another method by restricting the protein so that it became the factor which determined the rate of growth. They attempted to discover the concentration of

TABLE VI

Protein.	*Initial Body Weight. Grams.*	*Protein in Food. Per Cent.*	*Intake Per Gram of Gain.*		*Gain Per Gram of Food.*	*Gain Per Gram of Protein. Grams.*
			Food. Grams.	*Protein. Grams.*		
Lactalbumin ..	62	16.2	3.3	0.52	0.31	1.93
	61	"	2.6	0.42	0.39	2.41
	73	"	3.4	0.54	0.30	1.86
	67	"	3.8	0.60	0.27	1.67
Average			3.3	0.52	0.32	1.97
Lactalbumin ..	71	10.3	3.6	0.37	0.28	2.74
	63	"	4.0	0.42	0.25	2.41
Average			3.8	0.40	0.27	2.58
Lactalbumin ..	64	7.9	4.0	0.31	0.25	3.18
	70	"	4.5	0.35	0.22	2.83
Average			4.3	0.33	0.24	3.01
Lactalbumin ..	66	6.2	6.3	0.39	0.16	2.58
	67	"	6.0	0.37	0.17	2.69
Average			6.2	0.38	0.17	2.64
Lactalbumin ..	63	4.9	8.6	0.42	0.12	2.40
	63	"	8.9	0.43	0.11	2.32
Average			8.8	0.43	0.12	2.36
Lactalbumin ..	68	3.3	18.7	0.62	0.05	1.63
	67	"	24.6	0.82	0.04	1.23
Average			21.7	0.72	0.05	1.43

protein in the diet which promoted the greatest gain of body weight relative to the protein ingested. To accomplish this result they supplied the experimental animals with foods containing different percentages of the same protein. Using this method they secured data which is illustrated by the table on page 97.

In a similar study in which casein was the protein used, the protein intake was in most cases higher than in the lactalbumin experiments. For the sake of comparison the results are given in the table below in condensed form.

TABLE VII

Protein		Casein
Per cent in food		17.4
Weights of experimental animals		58-76 grams
Intake per gram of gain	Food	3.1; 4.0; 3.0; 3.5; 3.9; 3.1; 3.5; 3.0; 3.4. Average, 3.4.
	Protein	0.54; 0.70; 0.53; 0.61; 0.68; 0.53; 0.61; 0.52; 0.59. Average, 0.59.
Protein		Casein
Per cent in food		14.7
Weights of experimental animals		64-67 grams
Intake per gram of gain	Food	4.1; 3.5; 3.0. Average, 3.5.
	Protein	0.60; 0.51; 0.44. Average, 0.52.
Protein		Casein
Per cent in food		12.0
Weights of experimental animals		63-69 grams
Intake per gram of gain	Food	4.2; 3.5; 3.5. Average, 3.7.
	Protein	0.50; 0.42; 0.42. Average, 0.45.
Protein		Casein
Per cent in food		9.3
Weights of experimental animals		64-65 grams
Intake per gram of gain	Food	8.9; 6.4; 7.4. Average, 7.6.
	Protein	0.83; 0.59; 0.68. Average, 0.70.

Individual Variation in Growth of Rats.—From the data given it will be seen that under the same experimental conditions and on the same diet the variation in gain of body weight amounted to as much as 75 per cent and the gains per gram of protein ingested varied as much as 10 to 30 per cent in different animals. If we desire to learn the extent to which a food protein during growth can be converted into body proteins it would be more logical to accept only the greatest gain observed in any animal, discarding all other results, rather than to average the gains made by good and poor ones. From these data Osborne

and Mendel estimate that the gain of weight per gram of protein eaten during a period of eight weeks was for lactalbumin 2.34 and for casein 1.70 grams. In three experiments with casein fed at 12.0 per cent of the food mixture, the animals made an average gain of 2.25 gm. per gram of casein consumed. One animal attained a gain of 2.39 gm. and another of 1.99 gm. Their results are not very convincing as proof of the superiority of this method over other methods as a measure of the relative biological value of proteins.

Energy and Protein Used by Growing Animals.—The data of Osborne and Mendel discussed above have an interest quite apart from their worth as quantitative comparisons of the relative values of proteins. They support the view that "Economy of food can be effected only by supplying the young animal with as much as it will eat; economy of protein only by reducing the nutritive ratio below that at which the normal rate of growth can be maintained." This principle appears to be established, but it is quite another matter to determine whether or not economy of protein utilization obtained by the means just mentioned, makes for physiological well-being. The studies of McCollum and Simmonds, to be described later, point to the desirability, from the standpoint of the long maintenance of vigor, of a liberal but not excessive supply of protein in the diet.

The type of experiment which has thrown most light on the comparative values of the proteins of different foods is that first employed by McCollum and Davis (1915) for showing the nature of the dietary deficiencies of the cereal grains. Several investigators have applied their method to other important seeds and to leaves, tubers and roots, as well as foods of animal origin. The method consists in feeding to one group of animals a natural food as the sole source of nutriment, and to other groups the same food supplemented with single or multiple additions of purified foodstuffs (p. 32). The interpretation of the results was based on the number of food factors which had to be improved before growth could take place, and the number which, when improved in quality, could be shown to make the diet of better quality, as revealed by fertility, success in rearing young, span of life, and general well-being.

A procedure introduced in our laboratory (1919) for the study of the relative values of different proteins, or for the supplementary values of proteins from different sources, involves feeding a series of groups of animals on diets which are of uniformly

excellent quality with respect to all factors except protein. The diets contain 9 per cent of protein, which may be derived from a single food or from two or more sources. On such diets young rats are observed during the entire growth period and thereafter up to the time when pronounced developments of senile characters are noted. The groups contain animals of both sexes, and the fertility and infant mortality are carefully observed. The behavior of the animals, especially with respect to a tendency to destroy their new-born young when the protein supply is unsatisfactory, is also recorded.

This method was the outgrowth of considerable experience in searching for the most satisfactory procedure for making a comparison of the effectiveness of different foods as protein supplements for one another. McCollum and his co-workers had conducted numerous experiments in feeding pigs diets containing different percentages of protein. The work involved an effort to study the relative values of purified proteins and of the mixtures of proteins contained in individual natural foods, both for growth and for the replacement of the nitrogen lost through tissue metabolism (1911 and 1913). In the early studies too little was known about supplementing factors other than protein. None of these results are to be regarded as very satisfactory for the reason that factors other than protein in the experimental diets were not always suitably improved to make the results turn entirely upon the quality of the proteins of the food.

The above studies showed, however, that all the amino-acids necessary for the nutrition of an animal are contained in the proteins found in wheat, maize; and oat kernels. Certain ones are present in such limited amounts as to restrict the extent to which those more abundant can be utilized. For this reason these proteins are of relatively low value unless supplemented by proteins from other sources. With few exceptions the proteins of the cereal grains are of lower value than are those of milk and eggs, or than those of certain mixtures obtained by combining two or more of these seeds.

Quantitative Records of Food Consumption.—Many of our studies on protein values were criticized by Osborne and Mendel because when rats were employed no records were kept to show the amounts of food consumed by each individual. They pointed to the great differences in amount of food eaten by different rats as evidence that there must be a correlation between food consumption and growth. They were able, by using large amounts

FIG. 6.—Type of feeding device described on page 103.

of fat, to convert their food into a paste, which could be placed in a glass tube provided with a rod acting as a plunger. Each rat was kept in an individual cage. Food was from time to time expelled from the device into the feeding dish. They employed this device in their experiments and furnished figures of food consumption.

McCollum and co-workers were not able to secure a quantitative record of food intake for their rats until about 1915, for they employed diets ground to fine powder. Their plan did not admit of the inclusion of large amounts of fat in their food mixtures, because this procedure diluted so much the mineral content, protein content, etc., of any natural food under investigation. Large amounts of fats make it impossible to feed sufficient amounts of grains and legume seeds to give an accurate picture of their dietary properties. It was imperative, therefore, that rats should be fed with diets low in fats. A device was finally designed which left little to be desired for the quantitative feeding of such powders. It is possible to compare diets with respect to any component, *e.g.*, protein, vitamin, inorganic element, only when the diets have the same calorific value per gram, since animals eat for energy and not for protein or other element which may not be adequately supplied. The decision was made to use only diets poor in fats, since this allowed greater freedom in modifying natural foods for study. With this device it is easy to secure records of food intake which are accurate to within 1 per cent. Since the animals are not kept in individual cages, but in groups of four to eight in one large cage, most of their records showed the food consumption of the group and not of an individual.

Good nutrition can be secured with diets planned as just described, in which the proteins are derived solely from milk, kidney, or a mixture of certain seeds, notably of rye and flaxseed oil meal. So far as experience has progressed such effects cannot come from restricting rats to any diet containing less than 9 per cent of protein from any source. McCollum, Simmonds and Parsons (1921) classed as excellent any protein or protein mixture which would support nearly optimal nutrition over periods approximating two-thirds or more of the normal span of life of the rat when fed in amount corresponding to 9 per cent of the food mixture. Instead of trying as Osborne and Mendel had done to establish absolute values for the proteins, we only sought to compare any protein or mixture of proteins with some protein

which, according to the definition just given, would be classed as excellent.

In order to do this we confined four to six rats in a cage 24x24x20 inches in dimensions, screened on all sides, or on three sides with wire cloth of one-quarter inch mesh. The food records always showed the amount consumed by the group. Note was kept of the number of times, with dates, when the standard quantity of one kilogram of food was prepared. It was believed that a more detailed record of individual food consumption would add little value to the results or make their interpretation more significant. Exception must be made in experiments covering but a few days where the healing of rickets is being observed, since starvation causes confusing changes in bone structure. In rickets of a severe grade little rats become unable to walk effectively, and so do not find their way to the food.

Free Choice as Factor of Safety in Animal Nutrition.—Osborne and Mendel (1918) studied the ability of the rat to choose between adequate and inadequate diets. They offered two types of diets to rats and determined the food ingested. The diets differed in the nature of the proteins, and in one case in the vitamin B content. In one experiment one diet contained lactalbumin 18 per cent, and the other zein 18 per cent. The two rats employed ate considerably more of the lactalbumin food than of the zein food, but their choice resulted in nearly normal growth. In another experiment one diet contained 18 per cent of casein and the other but 4.5 per cent. The selection of food differed markedly in the case of the two animals. One animal ate about four-fifths of the high protein diet, the other ate more than 60 per cent of the food containing insufficient protein, and grew but little, whereas the rat which ate principally the protein-rich foods grew nearly normally. In a third experiment both diets contained 18 per cent of protein (casein), and the difference in the two diets lay in their vitamin B content, one containing natural protein-free milk, the other the artificial protein-free milk made of relatively pure milk sugar and salts. One rat ate about two-thirds of the former, and one-third of the latter mixture, and grew well. The other selected only the vitamin-poor diet during the first 20 days, and thereafter essentially only the vitamin-rich food. It failed to grow in the first period but responded when it changed to the complete food. In commenting upon these studies they say: "It is difficult to imagine what

the impulse is which led the rats to make these selections; for numerous experiments which we have made prove that the inferior foods offered to those shown in Charts II, III, and IV were entirely adequate to maintain them in good condition for very long periods. It is therefore interesting to have this evidence that the desire of a young animal for food is something more than the mere satisfaction of its calorific needs. The demands made by the growth impulse must also be met by a food of the proper chemical constitution."

Varying Food Consumption with Growth.—When a young animal is growing there is a constantly increasing need for food, and a progressive increase in consumption is observed. If two animals are fed diets which are alike except for their protein content, or the nature of their proteins, one may grow because the protein of its food is well constituted, while the other may fail because its protein is deficient in some respect. Comparison of food intake and consequently of protein consumption is not practicable in such animals, since the difference in size is becoming constantly more pronounced. Osborne and Mendel (1914-1915) have made an elaborate study of food consumption in rats under different dietary restrictions, and their data are of great interest in connection with the present question.

They found that rats growing well on a diet containing 9 per cent of casein when a small amount of cystin was added, frequently ate no more food in terms of calories, and ingested no more protein than did rats which were growing no better on even larger percentages of casein without the addition of cystin. They concluded in this instance: "It thus appears that a marked deficiency in any essential ingredient of the diet does not lead to a corresponding compensatory increase in food intake."

Their study of the rôle of appetite in regulating food consumption led them to state: "The experience which we have gained in measuring the food eaten by many rats at all periods of their growth has given us the conviction that the intake of the individual is determined in large measure by the energy requirement at any given period" (1914), (p. 358). To quote from page 359 of the same paper, "At any rate the animals do not consume *proportionately* more of the artificial ration because it happens to be decidedly poor in protein; but roughly speaking, they appear to limit their feeding to the amount of food yielding approximately the requisite energy." This view is in accord with that of Rubner (1902), who found that food which a dog would

eagerly devour at the temperature of 0° C, he would in part refuse when the temperature is raised to 33°, under which conditions his energy requirements are much lower.

In a later publication Osborne and Mendel (1918) reported the food consumption of rats fed diets containing varied amounts of fat. Since fat has about double the energy value of protein or carbohydrate, much smaller amounts of the fat-rich diets would furnish the required amount of energy than would be necessary with the fat-poor diets. In all cases food consumption was found to be regulated by energy needs.

McCollum and his co-workers have throughout their studies employed diets low in fats, and very much alike in their energy values. Food intake should have been, therefore, according to Osborne and Mendel's experience, entirely comparable. Numerous records obtained in recent years in our laboratory have shown clearly that this is the case. Quoting again from the paper of Osborne and Mendel (1918) (p. 523) "McCollum does not state the amount of food which his experimental rats ate; but if we assume that they ate quantities of the type of food which he employed in amounts approximately equal to those eaten by rats in our experiments on foods of corresponding calorific value, the difference between the results of his experiments and ours disappear." The force of their repeated criticisms of the work of McCollum vanish with the recording of this comment.

Owing to faults in the technic employed in earlier experiments, we find ourselves to-day with extremely little information concerning the manner and extent to which any purified proteins supplement one another. On the other hand we have a very considerable amount of accurate knowledge concerning the supplementary dietary relations among certain of our important food-stuffs.

Studies on Supplementary Protein Values.—With a method described above, in which an effort was made to determine the extent to which any protein or mixture of proteins falls short of the best quality yet observed, McCollum, Simmonds and Parsons made an elaborate study of a long list of simple combinations of two natural foods, supplemented with respect to all factors other than protein. In all cases the protein constituted 9 per cent of the diet since this plane of intake is a critical one even when the protein is of good quality. The life histories of experimental rats confined to such diets show clearly whether the

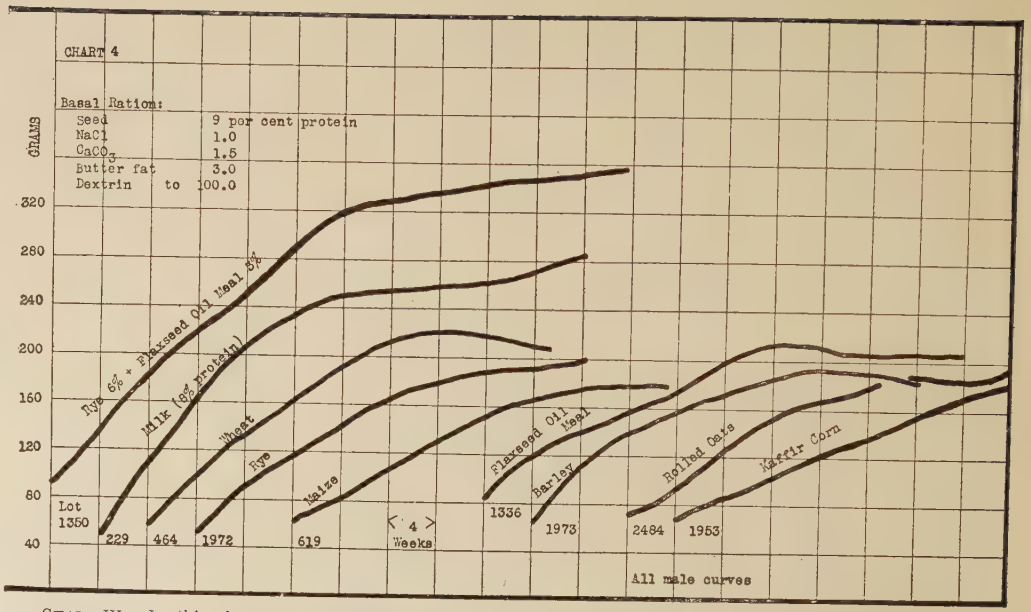


CHART IV.—In this chart are shown a series of growth curves of rats which were fed on diets which were comparable in every respect except in the quality of their proteins. All the diets contained 9 per cent of protein except Lot 229, which contained but 8 per cent. It will be seen that rye and flaxseed oil meal furnish a combination of proteins of extraordinary nutritive value, for growth was rapid and the ultimate size attained was great. When the remaining curves are compared it will be seen that 9 per cent of wheat protein produced growth a little less satisfactory than did 8 per cent of milk proteins. None of the other proteins were as good as those mentioned. The steepness of the curve denotes the excellence of the proteins.

Simmonds had reported severe kidney lesions in rats fed high protein diets (casein plus liver), and since others had not been able to confirm this work, using casein alone Newburgh and Curtis studied the effects of feeding high protein from several sources. They also gave large single injections of individual amino-acids and brought to light an important fact. They employed diets containing 12, 18, 25, 32, 39, and 75 per cent. of protein. They found that although casein is slightly injurious at high levels the injury may be easily overlooked. Beef muscle or liver fed at high levels produced pronounced kidney injury. Beef muscle produces albuminuria of about ten times the intensity as the same percentage of casein, and beef liver proteins are often more injurious than muscle. No animal on the liver diet survived a year. A diet containing 70 per cent. of beef muscle fed more than a year caused a diffused connective tissue increase around the glomeruli. Kidneys of rats receiving liver were without exception granular.

These investigators emphasize that the kind of protein and the time factor are the important ones in producing experimental lesions of the kidney due to high protein feeding.

The extensive nutrition studies on animals seem to place in a new light the older literature relating to human nutrition. There is no instance in our experience where a diet satisfactory in all other respects, but supplying just sufficient protein of good quality to support growth at approximately the maximum rate to the full adult size, has been found to promote as satisfactory nutrition over the entire span of adult life as would the same diet containing a more liberal supply of the protein factor. It has been frequently assumed by students of nutrition that after growth has been attained the nutritive needs of the body for protein can safely be met by a dietary in which the protein content is lower than is essential for optimum growth. Of the numerous experimental data from the work of McCollum and Simmonds, none support this view. They all point to the conclusion that when the life history of the individual is considered, a generous protein ingestion or one allowing a fair margin of safety over the lowest percentage which just suffices to induce maximal growth in the young serves to maintain vigor for the longest possible period.

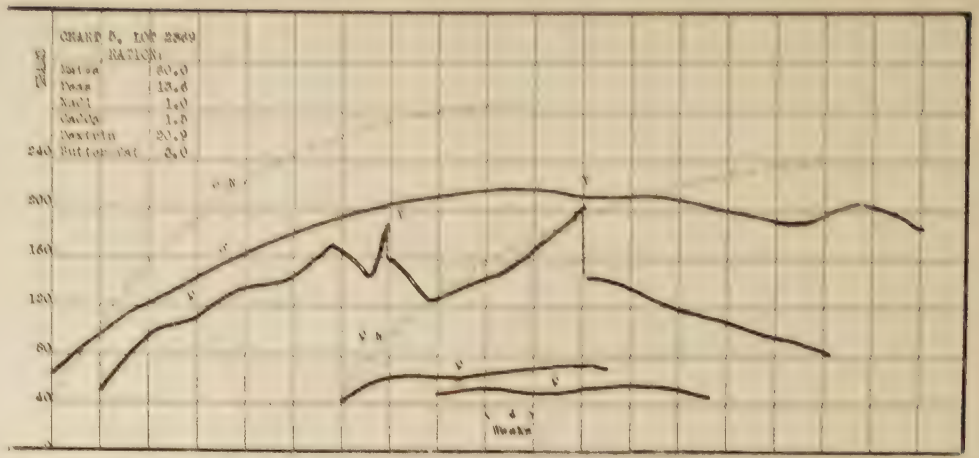


CHART V. This chart shows the amount of growth which it is possible to induce in a rat which is confined during growth to a diet containing but 9 per cent of protein derived from maize and peas. All the deficiencies of a mixture of maize and peas were made good by the addition of the inorganic salts and butter fat. The growth was stunted, and fertility low. The young born of the rats which had grown on this diet were never able to grow after they were weaned, and lived only five to six months. This chart should be contrasted with Chart VI in which the diet was essentially similar, except that the proteins were derived from a mixture of wheat and peas instead of maize and peas. The former mixture gives proteins of much greater nutritive value than does the latter combination. It is a matter of great practical importance to so combine our natural foods as to secure protein mixtures of high biological value. (See Table XII, p. 116.)

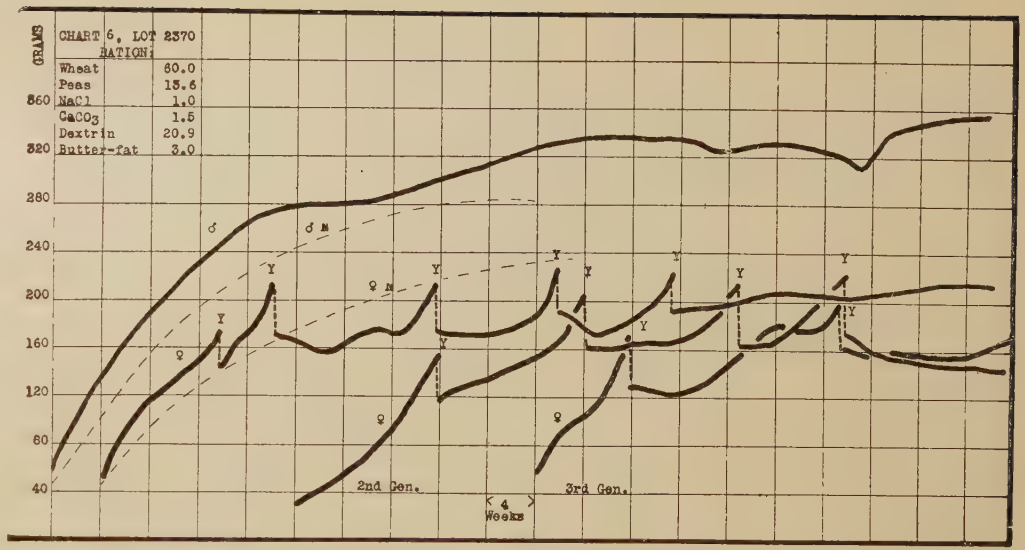


CHART VI.- The curves in this chart should be compared with those in Chart V. The two diets were alike in their biological value except in the quality of their proteins. Lot 2370 derived its proteins from wheat and peas and were able to grow well and showed high fertility because this combination contains proteins which supplement and enhance each other. Maize and pea proteins do not, on the other hand, enhance each other's values when they are combined. In animal production as well as in human nutrition, it is very important that the proteins of the diet have a high biological value

pea ration just mentioned, proved almost a failure for the nutrition of the rat. The animals confined to this diet, the only fault of which lay in its protein moiety, failed to grow as rapidly as they should, and remained stunted. Fertility was very low, and the second generation increased very slowly in weight. See Charts V and VI.

It would take us too far afield to comment in detail upon the relative merits of all the numerous protein mixtures which were included in this study. Some of these are tabulated in condensed form in Tables VIII, X and XII. The most striking thing brought out by these experiments is that in certain cases animal tissues are more effective for enhancing the value of cereal proteins than are the proteins of milk. A mixture of rolled oats supplying 6 per cent and milk supplying 3 per cent of protein was inferior to a similar combination of oats and liver, but about equal in value to mixtures of oat proteins and beef muscle or beef kidney, where the cereal furnished two-thirds of the total protein and the animal tissue the remainder. Barley and milk proteins likewise proved inferior to barley and animal tissue proteins. Wheat and milk, while very good as a source of proteins when combined in the proportions adhered to in these experiments, was inferior to wheat and animal tissues. Soy bean or pea proteins appeared to be about as well enhanced by milk proteins as by the three animal tissues studied.

Buckwheat Sensitizes Albino Animals to Light.—Tappeiner and Jodlbauer (1905) found that albino animals fed buckwheat and subsequently exposed to sunlight died within a short time, whereas colored animals did not suffer under the same conditions. They found that alcohol extracts a fluorescent dye from buckwheat, and that this when administered sensitized the animals to light, as did the buckwheat. This sensitization of white animals to light by the fluorescent substance or buckwheat has been repeatedly observed. Matsuyma (1923) found 10 per cent of buckwheat proteins gave satisfactory growth in rats. He found the seed to be rich in the diamino-acids.

Supplementary Relationships Among Feeding-stuffs.—Hart and Steenbock (1920) kept the cereal protein constant and varied the milk protein content of the diet in a series of experiments. They found that a highly efficient protein mixture is not attained until the proportion of liquid milk to corn meal reaches 1:1. In this proportion the milk nitrogen constituted approximately

(Continued on page 118.)

TABLE VIII

SHOWING THE EFFECT ON THE GROWTH OF THE NURSING YOUNG OF DIFFERENT QUALITY OF PROTEIN IN THE MOTHER'S DIET

No. of Experiment	Wt. of Mother After Birth of Young. Grams.	Wt. of Mother 10 to 22 Days Later.		Wt. of Mother at End of Nursing.		No. of Young.	Weight of Young at Different Ages.					
		Days.	Grams.	Days.	Grams.		Days.	Grams.	Days.	Grams.	Days.	Grams.
2369 *	144	18	150	46	120	4	18	71	31	107	46	123
2069 †	215	10	232	22	205	4	10	100	22	197	..	...
2370 ‡	192	12	170	37	170	5	12	74	33	160	37	175
2153 §	175	15	182	28	192	5	15	137	28	277	..	...
2365 ‖	173	18	157	45	146	5	18	70	30	102	45	210
2186 ¶	212	22	195	33	195	6	14	125	22	184	33	280
2767 **	270	19	275	29	270	6	19	163	29	330	..	...
2370 ‡	177	20	145	44	175	7	12	81	33	167	44	250
1236 ‡‡	142	..	...	40	146	7	16	133	30	286	40	544
2186 §§	215	17	213	45	215	9	11	123	17	153	45	382
2811 ‖‖	205	19	225	28	215	10	19	225	28	387	..	...

* Diet contained 9 per cent of protein from maize and peas. (Formula on p. 113.)

† Diet consisted of milled cereal products, legume seeds, beefsteak, a salt mixture and cabbage. (Formula p. 113.)

‡ Diet contained 9 per cent of protein from wheat and peas. (Formula p. 113.)

§ Diet derived its protein from milled cereals, beefsteak and legume seeds. (Formula p. 113.)

‖ Diet derived its 9 per cent of protein from barley and navy beans. (Formula p. 113.)

¶ Diet contained 9 per cent of protein derived from wheat and beefsteak. (Formula p. 113.)

** Diet derived its protein from whole cereal grains, legume seeds and casein. (Formula p. 113.)

‡‡ A high protein diet in which the protein was furnished by 50 per cent of beefsteak. This was supplemented so as to make good all its dietary deficiencies. Note the rapid and vigorous growth of the young. The formula for this diet is given on p. 113.

§§ Diet contained 9 per cent of protein derived from wheat and beefsteak. Although the protein content was low, the quality was so high that the young made remarkable growth. (Formula of diet, p. 113.)

‖‖ Diet contained a liberal amount of protein derived from cereal grains, legume seeds, casein and milk. Note the remarkable growth of the young during the nursing period. (Formula of diet, p. 113.)

TABLE IX

Composition of Diets Described in Table VIII

No. of Exp.	Wheat, Grams.	Maize, Grams.	Barley, Grams.	Rolled Oat, Grams.	Bolted Wheat Flour, Grams.	Corn Meal,* Grams.	Rice, Grams.	Pea, Grams.	Navy Bean, Grams.	Beefsteak, Grams.	Cabbage,† Grams.	Dextrin, Grams	NaCl, Grams.	$CaCO_3$, Grams.	Butter Fat, Grams.	Casein, Grams.	Milk Powder,‖ Grams.	Cod Liver Oil, Grams.
2369		60.0						13.6				20.9	1.0	1.5	3.0			
2069				8.0	30.0	15.5	8.0	8.0	8.0	10.0	10.0		1.0	1.5				
2370	60.0							13.6				20.9	1.0	1.5	3.0			
2153				9.5	30.0	19.5	9.5	9.5	9.5	10.0			1.0	1.5				
2365			50.0						13.6			30.9	1.0	1.5	3.0			
2186	60.0									4.1		30.4	1.0	1.5	3.0			
2767	30.0	19.5		9.5			9.5	9.5	9.5				1.0	0.5		10.0		1.0
2370	60.0							13.6				20.9	1.0	1.5	3.0			
1236 ‡										50.0		43.5	1.0	1.5	3.0			
2186	60.0									4.1		30.4	1.0	1.5	3.0			
2811	25.0	11.5		9.5			9.5	9.5	9.5				1.0	1.5	3.0		10.0	

* Degerminated corn meal.
† Cabbage desiccated, steamed and dried.
‡ Contained 1.0 per cent of KCl.
‖ Merrill-Soule Company's "Klim."

TABLE X

Supplementary Relations Between Proteins from Different Sources

ALL DIETS CONTAINED 9 PER CENT OF PROTEIN, TWO-THIRDS OF WHICH WAS DERIVED FROM THE VEGETABLE, AND ONE-THIRD FROM THE ANIMAL TISSUE OR FROM MILK.

No. of Experiment.	*Sources of Protein.*	*Observations on Growth, Fertility, Infant Mortality.*
2193	Wheat 60, kidney 4.2 per cent.	Growth excellent; reproduction good; infant mortality low.
2191	Navy beans 27.2, kidney 4.2 per cent.	Growth fair; far superior to growth on 9 per cent of bean protein.
2179	Wheat 60, liver 4.1 per cent.	Growth good; high fertility but very high infant mortality.
2177	Navy beans 27.2, liver 4.1 per cent.	Same as Lot 2191.
2182	Rye 50, liver 4.1 per cent.	Growth excellent; fertility good; infant mortality high.
2176	Peas 27.2, liver 4.1 per cent.	Growth fair; far superior to that on 9 per cent of pea protein.
2181	Oats 40, liver 4.1 per cent.	Growth good; infant mortality high.
2188	Oats 40, beef muscle 4.1 per cent.	Growth not so satisfactory as Lot 2181; fertility good; infant mortality 100 per cent.
2189	Rye 50, beef muscle 4.1 per cent.	Growth good; about like Lot 2182; fertility good; infant mortality high.
2186	Wheat 60, beef muscle 4.1 per cent.	Growth excellent; reproduction good; infant mortality high.
2387	Oats 40, milk powder 9.3 per cent.	Growth good; inferior to Lot 2181; similar to Lot 2188; fertility good; infant mortality high.
2391	Barley 50, milk powder 9.3 per cent.	Growth fair; inferior to barley and liver, kidney or muscle.
2389	Soy bean 16.6, milk powder 9.3 per cent.	Similar to results with rations of soy beans with animal tissues.
2390	Peas 27.2, milk powder 9.3 per cent.	Similar to Lot 2176; results same as with peas with kidney or beef muscle
2384	Wheat 60, milk powder 9.3 per cent.	Results not so good as with wheat with animal tissues.

The proteins of seeds are supplemented and enhanced better by animal tissues (meats) than by milk proteins. In all cases milk proteins enhance the proteins of seeds, however, and milk has the advantage of greater superiority as a supplement in respect to inorganic elements and is far superior to muscle meats as a source of vitamin A. (For further details see Charts showing growth curves.)

TABLE XI

Composition of Rations Described in Table X

No. of Exp.	Wheat Grams.	Oats Grams.	Rye Grams.	Barley Grams.	Navy Bean Grams.	Soy Bean Grams.	Pea Grams.	Kidney Grams.	Liver Grams.	Beef Muscle Grams.	Milk* Powder Grams.	Dextrin Grams.	NaCl Grams.	$CaCO_3$ Grams.	Butter Fat Grams.
2193	60.0							4.2				30.3	1.0	1.5	3.0
2191					27.2			4.2				63.1	1.0	1.5	3.0
2179	60.0								4.1			30.4	1.0	1.5	3.0
2177					27.2				4.1			63.2	1.0	1.5	3.0
2182			50.0						4.1			40.4	1.0	1.5	3.0
2176							27.2		4.1			63.2	1.0	1.5	3.0
2181		40.0							4.1			50.4	1.0	1.5	3.0
2188		40.0								4.1		50.4	1.0	1.5	3.0
2189			50.0							4.1		40.4	1.0	1.5	3.0
2186	60.0									4.1		30.4	1.0	1.5	3.0
2387		40.0									9.3	45.2	1.0	1.5	3.0
2391				50.0							9.3	35.2	1.0	1.5	3.0
2389						16.6					9.3	68.6	1.0	1.5	3.0
2390							27.2				9.3	58.0	1.0	1.5	3.0
2384	60.0										9.3	25.2	1.0	1.5	3.0

* Half skimmed milk powder. Merrill-Soule Company's "Klim."

TABLE XII

SUPPLEMENTARY RELATIONS BETWEEN PROTEINS FROM DIFFERENT SOURCES. (VEGETABLE PROTEINS ONLY.)

ALL DIETS CONTAINED 9 PER CENT OF PROTEIN. TWO-THIRDS OF THE PROTEIN WAS DERIVED FROM THE FIRST NAMED, AND ONE-THIRD FROM THE LAST NAMED FOOD-STUFF, EXCEPT LOTS 2346, 2344 AND 2345 IN WHICH EACH GRAIN FURNISHED HALF THE TOTAL PROTEIN.

No. of Experiment.	*Source of Protein.*	*Observations on Growth, Fertility and Infant Mortality.*
2367	Peas 27.2, Navy beans 13.6 per cent.	Very slow growth; permanent stunting. True of all combinations with legume seeds.
2383	Navy beans 27.2, soy beans 8.3 per cent.	Same as Lot 2367.
2346	Wheat 45, rolled oats 30 per cent.	Good growth; fertility low; long nursing periods; young inferior.
2344	Wheat 45, maize 45 per cent.	Growth fair; not so good as Lot 2346. Fertility low and nursing period long. Young inferior.
2345	Maize 45, oats 30 per cent.	Growth about like Lot 2346. Fertility high and infant mortality high.
2381	Barley 50, soy beans 8.3 per cent.	Good growth; fertility low; young inferior.
2378	Wheat 60, soy beans 8.3 per cent.	Same as Lot 2381.
2365	Barley 50, navy beans 13.6 per cent.	Good growth; fertility good; long nursing periods; infant mortality high.
2380	Oats 40, soy beans 8.3 per cent.	Fair growth; not so good as Lot 2381; fertility low.
2369	Maize 60, peas 13.6 per cent.	Fair growth; not nearly so good as Lot 2370. Fertility low. Nursing periods long. Young inferior.
2372	Oats 40, peas 13.6 per cent.	Good growth; much better than Lot 2369. Not so good as Lot 2370. Nursing periods long. Mortality high.
2370	Wheat 60, peas 13.6 per cent.	Very good growth; fertility good; infant mortality quite high. Nursing periods long. This combination is the best of all cereal and legume mixtures studied.

Cereal grains enhance each other's proteins better in all cases than do combinations of proteins of two legume seeds. Neither of the above are equal in the quality of their proteins to certain combinations of a legume seed with a cereal in which the cereal furnishes two-thirds and the legume seed one-third of the total protein diet.

In Tables X and XII the diets were satisfactorily constituted with respect to all factors other than proteins, so the results of the experiments described turned entirely on the biological value of the proteins of the rations. The formulas for the rations used in these experiments are given in full on pages 113 and 117.

TABLE XIII

COMPOSITION OF THE DIETS DESCRIBED IN TABLE XII

No. of Experiment.	*Wheat grams.*	*Maize grams.*	*Oats grams.*	*Barley grams.*	*Peas grams.*	*Navy Beans grams.*	*Soy Beans grams.*	NaCl *grams.*	$CaCO_3$ *grams.*	*Dextrin grams.*	*Butter Fat grams.*
2367					27.2	13.6		1.0	1.5	53.7	3.0
2383						27.2	8.3	1.0	1.5	59.0	3.0
2346	45.0		30.0					1.0	1.5	20.5	3.0
2344	45.0	45.0						1.0	1.5	5.5	2.0
2345		45.0	30.0					1.0	1.5	20.5	2.0
2381				50.0			8.3	1.0	1.5	36.2	3.0
2378	60.0						8.3	1.0	1.5	26.2	3.0
2365				50.0		13.6		1.0	1.5	30.9	3.0
2380			40.0				8.3	1.0	1.5	46.2	3.0
2369		60.0			13.6			1.0	1.5	20.9	3.0
2372			40.0		13.6			1.0	1.5	40.9	3.0
2370	60.0				13.6			1.0	1.5	20.9	3.0

30 per cent of the total nitrogen of the ration. They showed (1919) that meat crisps or fish meal, fed with starch and serving as the sole source of nitrogen, showed efficiency of but 40 per cent, whereas milk protein used under similar conditions showed a value of 60. The protein mixture from corn and milk, or oat and milk, showed production values of 60-65 per cent, with approximately 25 per cent of the nitrogen derived from skim milk, while the efficiency of the corn and tankage protein mixture, or a barley and tankage protein mixture was about 40 per cent. Their results show that "either milk or meat will supplement the proteins of the various cereal grains to approximately the same degree."

Hart and Humphrey (1916 and 1921) have made extensive studies of the supplementary relations of the proteins of various concentrates with the proteins of alfalfa and corn silage, and also the supplementary relations of the proteins of barley with alfalfa and corn silage. Their studies were made upon dairy cows, and efficiency of these proteins for milk production was observed. Hart, Steenbock and Humphrey (1920) found a ration composed of 7 parts of rolled oats and 7 parts of oat straw was an inadequate diet for cows.

Osborne and Wakeman (1920) have studied the proteins of the spinach leaf. Osborne, Wakeman and Leavenworth (1921), and Miller (1921) described the different forms of nitrogen compounds which exist in the alfalfa plant. Osborne and Mendel (1920) found that a mixture of skim milk and yellow corn supplemented by some green food was an adequate diet for young animals, although it was necessary to add calcium carbonate and sodium chloride to induce rapid growth.

Macy and Mendel (1920) fed rabbits, guinea pigs and pigeons on cotton-seed kernel and found it to produce injury, the most characteristic clinical symptoms of which were emaciation, loss of appetite, weakness, rough hair and unkempt appearance, disturbance in breathing, and finally coma and perhaps paralysis.

Macy and Alter (1921) fed rabbits, guinea pigs, pigeons and mice on cotton-seed meal and kernels supplemented with minerals and the necessary vitamins. The animals declined and died. Lesions were found in the kidneys.

Osborne and Mendel (1913 and 1915) state that egg white gave adequate growth in rats when they were given 18 per cent of the protein. Bond (1922) reported excellent growth in rats on diets in which 20 per cent of egg white formed the sole source

of protein. Boas (1924) found that 20 per cent of egg white, a dried preparation of Chinese origin, was inadequate as the sole source of nitrogen for the growth of rats. Bond and Boaz employed the same sample of egg white but secured different results. For some years there have been conflicting reports concerning the digestibility of raw egg white. The most recent investigation on this subject has been made by Rose and MacLeod (1920). They found the coefficient of digestibility for cooked egg white to be 82 to 86 per cent, for unbeaten raw whites 71 per cent, for beaten raw whites 83 per cent, and for half beaten raw whites 81 per cent. There was a decreased digestibility of about 11 per cent when the whites were not beaten.

Nevens (1921) states that in rations containing 10 per cent of crude protein furnished by cotton-seed meal, alfalfa hay, or corn, the utilization of the protein for growth of rats was 66, 62 and 49 per cent respectively.

Cajori (1921) reports that rats grew at a moderate rate on a diet containing proteins from the pecan. He states that tannin in this nut is a limiting factor.

Studies on Proteins of Legume Seeds.—Sure (1920 and 1921) has studied the nature of the deficiencies of the proteins of certain legume seeds. He found that arachin, a protein from the peanut, does not supplement the proteins of the Georgia velvet bean. Prolin alone, or prolin together with cystin and tryptophan did not improve the proteins of the velvet bean. He supplemented velvet bean proteins in one case with gliadin, in another with zein and in another with gelatin (1923). None of these improved the protein mixture markedly. On the addition of cystin to each of these combinations enhancement was marked only in the bean-gelatin combination.

Embrey (1921) found mung beans to supply enough protein for normal growth in mice. The protein of white kao-liang is better than that of red kao-liang, but neither seems to be adequate for growth. She found these beans to be deficient in calcium and sodium. She believes the Chinese persimmon is deficient in vitamins.

Mirande (1921) discusses lathyrism or intoxication caused by seeds of the chick-pea. The crushed seeds of Lathyrus sativus moistened with water and kept at 35 to 40° undergoes fermentation, liberating carbon dioxid and hydrogen sulfid. The hydrogen sulfid comes from the cotyledons and is completely extracted by water. Whole seeds do not give off hydrogen sulfid

when moistened. He believes the toxicity of the seeds to be due to the liberation of hydrogen sulfid in the digestive tract.

Johns and Finks (1920) state that proteins of phaseolus vulgaris, phaseolus lunatus and phaseolus angularis are deficient in cystin. Johns and Jones and their co-workers (1921) found the proteins of the yellow pea (Pisum sativum) did not require extra cystin, as was found to be the case with proteins of the genus phaseolus or the bean family. The same is true for the proteins of the lima bean (1922) and also the adsuki bean (1921). These authors (1924) have shown that the limiting deficiency of the lentil (Lens esculenta) is cystin. Finks and Johns (1922) showed that protein mixtures from the Chinese or Georgia velvet beans were inadequate for nutrition, and were not completed by adding cystin. These beans contain a toxic substance. Finks and Jones (1923) found the proteins from the palm kernel were adequate for normal growth when the meal constituted 80 per cent of the diet, and furnished 15.5 per cent of protein.

Jones and Finks (1921) found that bread made from 25 per cent of soy bean flour and 75 per cent of wheat flour, or from 15 per cent of soy bean flour and 85 per cent of wheat flour, supplied proteins and vitamin B in amounts adequate for the normal growth of rats. The mixed proteins were found to be 2 to 3 times as efficient for growth as wheat proteins alone.

Finks and Johns (1921) report that a diet containing only the proteins of tomato press seed cake supported normal growth in mice when the protein constituted 17.2 per cent of the food.

Sure (1921) sought to find what amino-acid in zein causes it to enhance the proteins of the pea. He confirmed the findings of McCollum, Simmonds and Pitz that zein supplements pea proteins (Vicia sativa), whereas lactalbumin and gelatin do not. He showed that neither arachin nor edestin (hemp-seed) improves the quality of pea proteins. Sure (1921) further demonstrated that no improvement was seen in pea proteins when these were supplemented with leucin, an amino-acid, or with the "leucin fraction" which contains leucin, valin and alanin, all of which are digestion products of proteins. Tyrosin alone or with leucin and cystin did not enhance pea proteins, so he drew the conclusion that "the benzene ring must be eliminated as the nucleus responsible for the deficiency of the proteins in question." He also states (1921) "Prolin, even in the absence of

alanin, leucin, valin, cystin and tyrosin, did not supply the missing link furnished by zein in improving the poor quality of the proteins of the pea." These experiments eliminate alanin, leucin, valin, tryptophan, lysin, cystin, tyrosin and prolin as being the amino-acids which are responsible for the deficiency of the proteins of the pea. Sure says "Since zein, which supplements the pea, is very low in arginin and histidin, it is thought possible that zein may contain one or more amino-acids which we do not know about." Such studies as the foregoing illustrate how in time our knowledge of the specific deficiencies of the proteins of different foods are likely to be made clear.

Sure (1922) found that neither lactalbumin, arachin nor zein improve the dietary quality of edestin. Edestin fed at 12-18 per cent of the ration, even when carrying 2 per cent of the total nitrogen in the form of an alcoholic extract of wheat germ, did not suffice for growth. Edestin was enhanced by cystin only when accompanied by lysin. Sure (1922) using edestin, added cystin, lysin and arginin without marked enhancement. In another experiment with this mixture he added prolin and in another pyrrolidone carboxylic acid. He concluded that prolin is an essential amino-acid for growth, and that the animal has not the capacity to transform pyrrolidone into pyrrolidine carboxylic acid, or into prolin.

Salmon (1922) fed mature pigeons on dried ground velvet beans and found that on the second day all birds showed rough feathers and a sleepy appearance. Several died after 4 or 5 days. Plimmer and Rosedale (1922) found caseinogen and lactalbumin, when properly supplemented by other nutrient principles, to form a good source of proteins for growing chicks.

McCandlish and Weaver (1922) studied the comparative values of old process linseed oil meal, peanut meal, soy bean meal and wheat gluten as protein supplements in the rations of dairy cows. They found that cocoanut meal had a higher value than the others despite its low protein content. In palatability they found the feeds to range as follows: linseed oil meal, soy bean meal, gluten feed, peanut meal and cocoanut meal.

Mitchell and Villegas (1923) studied the utilization of proteins of cocoanut, corn and soy bean meal, and mixtures of these. The rations contained 5 or 10 per cent of protein. On a 5 per cent protein ration the average utilization of cocoanut meal protein was 77 per cent, of corn meal 72 per cent, and of soy bean 78 per cent. On a 10 per cent protein ration their results show

that cocoanut meal proteins are somewhat less efficient than are those of soy bean, while between soy bean and rice bran there was no clear difference. Very little supplementary effect was induced when mixtures of these proteins were fed. These investigators employed a nitrogen balance method of study and their experiments covered but a few days.

Maynard and co-workers (1923) found that combinations of peanut oil meal and rice bran are superior to corn meal proteins alone, but a mixture of corn meal and rice bran was even better. The supplementary relation of peanut meal and corn meal is not marked. Maynard employed the method of McCollum and Simmonds of feeding diets containing the critical content of 9 per cent of protein.

Shiba and Koyama (1923) found that rats grew less with soy bean protein than with peanut protein. Eddy and Eckman (1923) found that peanut flour proved slightly superior to meat for growth and markedly superior for reproduction, as a supplement to the proteins of wheat flour. Morgan and co-workers (1923) state that the proteins obtained from ground blanched almonds after removing the oil supported normal growth in mice when the protein constituted 17.2 per cent of the food.

Funk, Paton and Freedman (1923) found that treatment of neutral sodium caseinate with fuller's earth, and also oxidation with hydrogen peroxide or by heating in the air, resulted in the loss of a considerable amount of its nutritive value. Such casein when fed to animals produced malnutrition.

Hartwell (1923) believes that darkening of the coat of rats is brought about by feeding large quantities of proteins. Proteins rich in tyrosin and tryptophan are said to blacken the hair. She states "These experiments support the view that the melanins are formed from tyrosin and tryptophan. Mitchell and Carman (1924) studied the value of proteins of wheat, eggs and pork. The average biological values found for the proteins of eggs, pork and wheat at 8 per cent level of intake were 93, 74 and 67 per cent respectively. These figures were arrived at by short metabolism records of rats.

In this chapter an attempt has been made to make clear the types of experiments which have been employed for the study of the differences in the nutritive values of proteins from different sources, and to determine the exact nature of the limiting deficiencies of each. The experimental work on which this discussion is based constitutes the most confusing phase of the literature

of nutrition. This is true for the reason that, although the specific problems which have been investigated have in great measure been cleared up, the original papers have failed in a number of cases to clearly indicate how the data later recorded modified or rendered obsolete earlier observations made by the investigators themselves. Frequent references by persons who have made no thorough study of the literature to supposedly proven facts which were later disproved by other experiments, have convinced the authors that an interpretation was here especially desirable. This is very difficult to make in an entirely clear manner without exceeding the space which could be allotted to it.

CHAPTER VIII

THE PHYSIOLOGICAL REQUIREMENTS OF MAN FOR PROTEIN

The observations in the preceding chapters, together with the data presented in a forthcoming chapter, showing the profound damage which results from lack of sufficient of the essential mineral elements or of the vitamins, illustrate how complicated the problem is of interpreting quality in a diet. One can never safely focus attention upon a single factor and use this as a criterion of much significance. A great deal of detailed knowledge, both of the chemistry of foodstuffs and also of the nutritive needs of the body, is essential to the formation of safe judgments regarding any problem in nutrition.

With these data in mind we may turn to the studies which have been conducted upon human subjects and evaluate them anew in the light of newer observations on animals. Outstanding among nutrition studies on man are those of Chittenden (1904 and 1907) and of Benedict (1906). Since far-reaching deductions have been drawn from these results a few words of comment are called for in reference to their trustworthiness as a basis of guidance in deciding upon a safe dietary regimen for man.

Physiological Economy in Nutrition.—Chittenden conceived the idea that a low protein dietary, when it provides a suitable variety, will best meet the nutritive requirements of the body. He emphasized the desirability of relieving the organs of the task of degrading daily more protein than is necessary for the repair of tissue waste. He also emphasized the fact that the body does not tend to store protein which is ingested in excess of the daily need, and that an excessive protein ingestion promotes putrefactive decomposition in the intestine. This results in the absorption of products which are physiological abominations. The logical deduction was that the protein intake should be reduced to as low a level as will keep the body in nitrogen equilibrium.

In order to demonstrate whether such a regimen is safe for

the adult, Chittenden tested upon himself the proposition which had hitherto been accepted on the authority of Voit and of Atwater, viz., that the average adult at medium work requires about 116-120 gm. of protein per day. He reduced his protein intake to about one-third this amount and felt better and was clearer mentally than he had been on a more liberal protein dietary. A brief comment has already been given of the experimental demonstration which he planned and carried out during 1903-04 at Yale University, in which a group of faculty men, a group of students and a group of soldiers participated. During a period of nine months the men reduced their protein intake to about half the accepted requirements. There was no evidence that these men were not in excellent physical condition at the end of the period of observation. The deduction was drawn that such a dietary regimen appeared to be suitable for the maintenance of physiological well-being in the adult for an indefinite period.

The experiments of Benedict covered the period of a school year. The experimental subjects were volunteer college students. He was led to abandon his earlier view that a liberal protein dietary is best for the promotion of physical health, and to conclude that his experience with young men restricted for a few months to an abstemious diet low in protein warranted the acceptance of the dietary principles enunciated by Chittenden.

High Protein Dietary and Kidney Damage.—The view has long been held by many that the metabolism of large amounts of protein which necessitates the excretion of large amounts of metabolic end products might cause damage to the kidneys but no definite experimental evidence on this point was gained until recently. Squire and Newburgh (1921) discussed renal irritation in man on a high protein diet. Newburgh and Clarkson (1923) found that most rabbits which ate a diet containing 27 per cent or more of protein (meat) for 6 months or more, developed a chronic nephropathy. The lesion, they believe, may in part be caused by infection acting on a kidney made more susceptible to infection by diet. The injury, they suggest, may be due to excessive excretion of certain amino-acids, or to a combination of this and the acidity of the urine. The latter alone does not cause such injury. Drummond and co-workers (1922) fed a diet containing 83 per cent of casein, supplemented with other dietary essentials. Their rats grew at a subnormal rate and did not breed, but appeared to be in good health. The weights and

microscopic appearance of the internal organs were normal. Body fat was present but the ribs were beaded. Intestinal putrefaction was excessive. They fed kittens raw lean beef with yeast, a salt mixture and shark liver oil. Marked beading of the ribs but no other abnormality of the tissues was observed when examined microscopically. It seems probable that both their rats and kittens were suffering from what McCollum, Simmonds, Park and Shipley have designated as high phosphorus rickets, since their diets were excessively rich in phosphorus and poor in calcium. Their animals apparently did not live a sufficient length of time to produce kidney lesions. Polvogt, McCollum and Simmonds (1923) reported studies on rats which had been fed for periods varying from 250 to 400 days on diets containing 31 to 41.3 per cent of protein. They made a special point of having their diets satisfactory in their composition with respect to factors other than protein. All the animals had lesions of the kidneys of considerable severity. Even comparatively young animals of the fifth generation of a family confined to a high protein diet showed lesions. There was intense congestion and the formation of hyaline casts in large numbers. Osborne and Mendel (1921) conducted experiments using 90 per cent of protein in the diet which consisted of 5 per cent of inorganic salts and vitamins in the form of small daily doses of dried alfalfa and brewery yeast. Even with such foods almost devoid of carbohydrates the animals made very satisfactory growth to maturity. Osborne, Mendel, Park and Darrow (1923) fed rats high protein diets and noted hypertrophy of the kidneys but no nephritis was apparent. No inflammatory or degenerative changes were observed.

Newburgh and Marsh (1924) discussed renal injury resulting from high protein diets and suggested that it might at least in part be caused by the passage of unusually large amounts of some amino-acids through the kidney. In order to test this hypothesis, amino-acids were injected intravenously into rabbits and dogs. Glycocoll, alanin, leucin, glutamic acid, arginin and phenylalanin in doses up to 5 gm. per kilo caused no demonstrable injury. On the other hand, histidin, tyrosin and cystin in single injections of less than 1 gram per kilo regularly caused albuminuria and cylindruria even in dogs. Sections of the kidney showed acute parenchymatous injury. The results with tryptophan, aspartic acid and lysin are still in doubt. Serin and prolin have not been tested. This discovery is of extraordinary

interest in connection with the ability of the kidney to pass certain complexes derived in the normal course of digestion from protein without injury, whereas others exerted destructive action upon that organ.

The extensive nutrition studies on animals seem to place in a new light the older literature relating to human nutrition. There is no instance in our experience where a diet satisfactory in all other respects, but supplying just sufficient protein of good quality to support growth at approximately the maximum rate to the full adult size, has been found to promote as satisfactory nutrition over the entire span of adult life as would the same diet containing a more liberal supply of the protein factor. It has been frequently assumed by students of nutrition that after growth has been attained the nutritive needs of the body for protein can safely be met by a dietary in which the protein content is lower than is essential for optimal growth. Of the numerous experimental data from the work of McCollum and Simmonds, none support this view. They all point to the conclusion that when the life history of the individual is considered, a generous protein ingestion or one allowing a fair margin of safety over the lowest percentage which just suffices to induce maximal growth in the young serves to maintain vigor for the longest possible period.

CHAPTER IX

THE DIETARY PROPERTIES OF INDIVIDUAL FOOD-STUFFS

In preceding chapters an effort has been made to make clear what constitutes a satisfactory diet, and the type of experimental procedure by means of which this knowledge was gained. For the purpose of placing this knowledge in proper perspective and correlating it with the older views of problems in nutrition, a brief account of certain earlier investigations and the deductions made therefrom were presented. With the object of broadening the vision of the present day investigators so as to enable them and their successors in this field to appreciate how carefully experimental work must be planned and controlled in order to avoid conclusions which tend to confuse rather than clarify, a critical examination of some classic errors has been included.

It will be appreciated how confusing was the task of interpreting the cause of failure or of success with experimental diets, when it is recalled that a few years ago no definite conception was possible of the existence of several dietary factors which are indispensable in the food supply of man or certain animals. Fortunately for students in this field the rat does not require the vitamin C, so the element of freshness in food was not essential for success in feeding experiments. This species was, therefore, of special value in gaining a satisfactory working hypothesis regarding the number of essential factors in nutrition. Even with this species, however, in addition to the unknown factors there were several variables, any one of which determined success or failure in the development. These were the quality of the proteins and the amounts of certain mineral elements contained in the food mixture. The solution of such a problem demanded first the accumulation of a considerable amount of data based upon the outcome of experiments, each of which was not possible of interpretation except in the light of all the others. It must be admitted that success was actually in no small measure the result of active imagination and successful theoriz-

ing as to the true meaning of the effects of the experimental diets on the animals observed.

In order to understand how foods should be combined so as to make good each other's deficiencies, it is necessary to describe the dietary properties of each of the more important natural foods. This will form the subject of the present chapter.

Wheat.—This is the most important seed grain used as food by all western peoples, and is second only to rice in respect to the quantity used by man. Rice is in greatest favor among peoples in the wettest regions of the world. The dietary properties of wheat have been described in part in Chapter II in connection with the development of the biological method for the analysis of a food-stuff. The proteins of the grain are a little more efficient for the promotion of growth than are the same amounts of protein from milk or eggs. No cereal grain has been yet studied which contains proteins superior to those of wheat. Its prominent place in the diet of mankind is justified by the results of experiments on animals. The quality of wheat proteins is illustrated by the comparisons shown in Chart IV. The results of feeding diets in which wheat was the sole source of protein have not been uniformly successful, and it appears that some samples of wheat are superior to others. It is well known that the proteins vary markedly in different samples of wheat with respect to their glutinous property, which is so important in bread making. The reason for this has not been satisfactorily explained. It may be due in part at least to variability in the proportions among the several individual proteins in the seed. If this is true, it would account for such inequalities in nutritional value as have been observed.

Wheat is too poor in calcium to meet the needs of the growing rat. The average content of this element per 100 gm. of this cereal is about 0.041 gm. The optimum content of calcium for the growing rat is not far from 0.64 per cent of the food mixture. Phosphorus, sodium, chlorin and iodin are also present in the wheat kernel in amounts too low to support normal nutrition in this species.

The composition of grains varies according to the nature of the soil and the amount of water supplied, and also depends upon the manner of its application, especially under irrigation. Greaves and Carter (1923) conducted valuable studies on wheat, oats and barley. In different experiments water was applied (a) in large amounts a few times; (b) the same quantity was furnished in

small amounts at intervals. They state that large applications of water wash the nitrates beyond the roots and this results in low nitrogen content of the grain. The decrease for wheat was 21 per cent, for oats 40 per cent, and for barley 19 per cent. Where water was supplied in small, frequent applications the protein content of the grain was high.

The quantity of ash increased progressively as the irrigation water was increased. The increase amounted for wheat to 46 per cent, for oats 31 per cent and for barley 36 per cent. The increase in phosphorus was 55, 35 and 30 per cent for wheat, oats and barley respectively. The increase for calcium was 155, 22 and 41 per cent respectively for wheat, oats and barley. Similar increases were observed for potassium and magnesium. These results correspond with those of Forbes (1921).

McCarrison (1924) suggests the possible relation between fertility of the soil, aeration, atmospheric temperature, soil deterioration and water, and the vitamin content of plants. He also points out the well-known superiority of natural over artificial manures in influencing plant growth.

Bell and Mendel (1922) examined different wheats for their content of vitamin B. Marquis spring wheat had a higher vitamin content than Minnesota winter wheat.

Hart, Halpin and Steenbock (1920) fed half grown chickens on wheat, wheat gluten and calcium carbonate. They found the birds unable to live longer than 3 months. In contrast chickens fed corn meal, gluten feed (corn) and calcium carbonate made slow growth and maintained themselves.

Wheat Germ.—This constitutes 1.5 per cent of the entire kernel. It contains about 30 per cent of protein. The remainder of the seed, about 83 per cent, is endosperm containing about 11 per cent of protein.

McCollum, Simmonds and Pitz (1916) found wheat germ to be exceptionally valuable in several respects. Its proteins are abundant and are of good quality; its content of the essential mineral elements is much greater than in bolted flour; it is exceptionally rich in vitamin B and contains some vitamin A. The oil, of which the germ contains about 10 per cent, leaves a somewhat unpleasant after taste, and is somewhat injurious to animals. Evans and Bishop (1922) report experiments which seem to show the presence in the oil of a vitamin which they call vitamin X, which is essential for reproduction.

The germ contains a yellow pigment which contributes to the

color of whole wheat flour. The color of the latter is due principally to the bran layer. In making white flour the germ and the bran are both removed.

For some reason wheat germ as a stock feed has never found favor with farmers, and in recent times no effort has been made to market the pure product for feeding. It is mixed with bran or a low grade flour known as middlings, and reaches the feeder in this mixed form.

Osborne and Mendel (1919) stated that pure embryo separated from all other parts of the seed failed to promote growth, but supported maintenance when used as the sole source of vitamin B. Bell and Mendel (1922) reported that they could detect no difference between the vitamin B content of hand dissected and commercial germ.

Bolted Wheat Flour.—In point of quantity consumed white flour is the most important energy food in America and Europe. This is remarkable because it is notably deficient in more dietary factors than any other single food entering into the diet, except sugars, starches and fats which are marketed in the pure state. It consists essentially of starch, proteins and inorganic salts. Its proteins are of relatively poor quality, and its mineral components are conspicuously lacking in calcium, sodium, chlorin, iron and phosphorus.

Gliadin and glutenin are the two principal proteins of flour. These are unlike any proteins in the body in their yield of the amino-acids. These proteins are not constituted so as to enhance each other's value by compensating each other's deficiencies, and cannot be effectively transformed into body proteins unless they are combined with other foods wisely chosen. Wheat flour is very deficient in all vitamins.

The merits of flour as a food rest essentially upon the peculiar glutinous properties of its proteins, which make possible the formation of dough. This is tenacious and permits of leavening by the generation of carbon dioxid gas within it, through the agency of yeast or baking powder. By this means light, spongy bread is secured. Bolted flour has excellent keeping qualities and can be distributed without commercial hazard. This is a matter of great importance in a country like the United States. It will, despite its shortcomings from a dietary standpoint, remain our most important energy-yielding food.

It is not to be understood from what has been said that wheat flour is not a wholesome food. When properly combined with

such other foods which make a well proportioned diet, every factor of which is adjusted so as to meet the needs of the body, it becomes an entirely satisfactory part of the diet. The keynote to the discussion of the individual foods entering into the diet of man is the importance of using proper combinations of foods. Greig and Curjel (1918) found the "atta" biscuit which contains 15 per cent of wheat germ to be rich in vitamin B. They recommend these biscuits for emergency rations.

Ghose (1922) examined different Indian food-stuffs. He found bleached (pure white) Indian flour to be defective in vitamin B and crude "attah," which is a mixture of various flours, as wheat, peas, maize, etc., and unbleached Indian flour, to have considerable quantities of it.

The attitude which one should take toward the discussion of the relative merits of bolted as against whole wheat flour is now easy to understand and appreciate. The latter is decidedly more suitably constituted to maintain well-being for a short time if it serves as the sole article of diet, as it might under famine conditions. Whole wheat flour is an incomplete food and must be supplemented with other foods in order to compensate for its deficiencies. The unfortunate individual, who through faulty habits of living finds at an age at which he should be still in possession of the full vigor of middle life that his efficiency is diminishing and the joy of living slipping away, has not infrequently turned for relief to substituting whole wheat flour for the staple white variety. As a rule those who advocate this practice exhibit in some degree the spectral mien of the dyspeptic. They would gain much more through adhering to a diet well balanced than through adopting this or any other dietary whim or fad.

Wheat bran has been studied by Osborne and Mendel (1919). The proteins of this part of the kernel have a higher value than those of the germ, and they are distinctly superior to those of bolted flour. Bran is poorer in vitamin B than the germ.

Maize Kernel.—This cereal grain has dietary properties similar to wheat. Its flavor and physical properties, however, are different. Its proteins have no glutinous properties so it is not possible to form a dough with finely ground maize. In the southern United States maize is much prized as a human food. The United States Food Administration (1918) found in a dietary study that it constituted 23 per cent of the total food in-

take of Tennessee and Georgia mountaineers, and 32.5 per cent of that of southern negroes. Among 72 northern families in comfortable circumstances only 1.6 per cent of the diet was derived from this grain. The great bulk of the maize crop, which in this country amounted to three billion bushels in 1917, is used in stock feed.

When fed as the sole source of proteins, the proteins of maize have a slightly lower value in nutrition than have those of wheat. Its mineral deficiencies are about the same as those of the latter. These two grains are comparable in their content of vitamin B.

Steenbock and Boutwell (1920) observed that yellow maize is superior to white or red varieties as a source of vitamin A. They believed at one time that vitamin A was associated with the yellow pigment of plants. This view has been in great measure discarded.

Maize used as human food is now freed from its germ and its bran layer for the purpose of improving its appearance and more especially its keeping qualities. Maize germ, like wheat germ, is rich in oil and this tends to become rancid when the kernel is ground and exposed to the air. Both white and yellow grain are used for making meal for human consumption. Corn oil is now marketed as a salad oil.

Vitamin Content of Foods.—Much effort has been expended in recent years by a number of investigators to determine the vitamin content of different foods. It has seemed to us, from the results of our studies, that this matter has excited greater interest than its importance justifies. We found in numerous comparative studies that different samples of cereal grains differ considerably in their content of vitamin B. Steenbock's observations on the differences in vitamin B content of different samples of maize support the view that general statements as to the relative values of many of our common foods with respect to the vitamin content is all that are justifiable. The fact that we can readily prepare diets from ordinary wholesome foods which will contain several times the minimum amount of vitamins on which apparently normal nutrition can be maintained over considerable periods, tends to render the discussion about the advisability of taking concentrated commercial preparations of vitamins a purely academic one. Investigators are agreed that there is no need for commercial vitamin preparations.

Oats.—Rolled oats are used principally as a breakfast food.

It has long been a principal food grain in Scotland. The oat kernel is comparable to wheat or maize in its dietary properties in nearly all respects. It appears from the data available that there is much less vitamin A in rolled oats than in wheat and certainly much less than in yellow maize.

Oats have been a great favorite among feeders of horses, and are believed by many to have exceptionally high nutritive value because animals fed liberally with oats frequently show much spirit when driven or ridden. In our experience rats fed on restricted diets of certain types, faulty in one or more respects, become restless, irritable and apprehensive, and the thought occurs that perhaps the common expression among horsemen that an animal "feels his oats" may have been misinterpreted. The tendency of horses fed liberally on this grain to exert themselves greatly without urging may be the result of increased irritability and apprehensiveness. Scheunert (1918) fed a horse exclusively on oats. He noted that the calcium appearing in the feces was irregular from day to day and that there was a steady decrease in the urinary calcium as the experiment progressed. After 19 days, when the experiment was discontinued, the horse showed loss of appetite, was declining in weight and had a tendency to lameness.

Rice.—This is the most important cereal grain in the diet of more than half of the human race. It is used especially in the wettest parts of the world. It has never found much favor in the United States, but is used in small amounts. Among primitive peoples rice is eaten without polishing, in which form it is known as red rice, but it is usually treated so as to lose a large part of its germ. This loss results from the pounding of the kernels in rude mortars. The bran layer, which is richer in mineral salts than the endosperm of the seed, is retained in this process.

Rice which is used for export and for sale in the large cities at some distance from the place of production is polished by abrasion. The abrasive action results in wearing away the bran and germ. This mixture is known as rice polishings. The germ of rice, like that of wheat or maize, consists of cellular structures which are the seat of protoplasmic activity, and is a more complete food than any other part of the kernel. It contains almost all the fats found in the grain, and is more efficient in nourishing insects as well as higher animals than is the polished grain. Hamada (1923) reports that rice embryo protein has a high

nutritive value. Unpolished rice loses its flavor owing to the fats becoming rancid when kept for considerable periods in a warm climate. Polished rice can be handled without commercial hazard.

McCarrison (1923) concluded that vitamin A is present in paddy before it is milled. The milling of raw paddy does not remove the whole content of this substance so it is not confined to the peripheral layers of the grain. He states that it is destroyed in great measure by steam passing through paddy when it is contained in the vats, as in parboiling.

The practice of polishing rice had its origin in the desire to improve its keeping quality, and the incidental whitening of the kernels has led to the establishment of a demand for a white product. *This, and the artificially established liking for white flour and white corn meal, is an illusrtation of the failure of the instinct of man to serve as a safe guide in the selection of food.* The esthetic sense is appealed to in greatest measure in this case by the products of lowest biologic values.

Attractiveness of rice to the eye is so important a factor commercially that the practice of artificial whitening of the polished kernels has come into vogue. This is accomplished by coating the kernels with talcum powder, the latter adhering by means of a thin coating of glucose. The milky appearance of the water in which rice is washed is due to the talcum remaining in suspension. Rice which has been polished but not coated in this way is called brown rice as contrasted with the coated or white rice.

Chart III shows that there are four dietary factors in which polished rice is of such poor quality as to require improvement before it becomes a complete food. Its proteins are of low value. It is too poor in all essential mineral elements to meet the needs of a growing animal, and is nearly free from vitamins A and B. The data in Chart III were obtained with the rat and do not bring out the fact that rice is lacking in the vitamin C. This substance is not essential in the diet of the rat.

Kennedy (1924) found wild rice to contain a higher percentage of protein than most other cereals, but it resembles the cereals in containing proteins of rather low biological value. It also resembles other cereals in containing inorganic material unsuitable for the promotion of growth. Its content of vitamin A is low, but it contains a sufficient amount to prevent xerophthalmia. Wild rice has a greater food value than the cultivated polished

rice, because its proteins are of better quality. It contains adequate amounts of vitamin B for growth.

Barley, Rye and Millet.—Steenbock (1918) and Osborne and Mendel (1919) have reported biological analyses of these cereals. They possess essentially the same dietary properties as do those already described. Steenbock (1923) states that the vitamin content of millet is not uniform. He questions whether the vitamin content can be correlated with the size relation of the germ to the endosperm. It also appears that among millets the vitamin A content does not bear any definite relation to total pigment. His conclusions are at variance with those expressed by McCollum, Simmonds and Pitz (1917), who observed fairly good growth in rats which derived all their vitamins A and B from 25 per cent of millet seed in the diet. They suggested that since the millet seed is very small probably the germ constituted a large proportion of the kernel and so accounted for the observed relatively high content of vitamin A in this seed.

Peas and Beans.—These seeds belong to a class of plants known as legumes, which are able to utilize the nitrogen of the air for the purpose of synthesizing proteins during growth. Other plants have to secure their nitrogen from the soil in the form of nitrates or ammonium compounds. The legume seeds are richer in protein than are any of the cereal grains. Wheat, maize, oat and rice kernels contain about 11, 10, 15 and 9 per cent respectively, whereas peas and beans contain about 23 per cent of protein. For this reason the latter were long regarded as especially valuable from the standpoint of protein. Beans have been frequently spoken of as "poor man's meat." Unfortunately, modern experimental studies have not lent support to this view. Their proteins appear from the results of chemical analysis to be much more adequately constituted than are those of wheat or maize, but nutrition experiments on animals show clearly that there is something lacking in their molecules which limits the extent to which they can be converted in the body into tissue proteins.

The foods next in importance to the cereals in the prevention of beri-beri are pulses. Grijns (1901) and Hulshoff-Pol (1907) demonstrated the value of certain beans (Katjang-hidjoe) in the prevention and cure of human beri-beri and avian polyneuritis. Johns and Jones and their co-workers (1915 to 1921) have made extensive studies of the legume seeds. Reed and Sure (1921-1923) found the Georgia velvet bean to be injurious to rats

when 40 per cent was included in the diet. These beans are rich in vitamins A and B. Ghose (1922) found Indian lentils to be a good source of vitamin B. Findlay (1924) said samples of Indian lentils and peas kept for 38 years still contained appreciable amounts of vitamin B. Seeds which had lost their power of germination have not necessarily lost their vitamin B content. He reports that Jansen found rice stored as paddy for 100 years to have lost little or none of its anti-beri-beri vitamin.

Osborne and Mendel (1912) were unable to secure growth with pea and bean proteins when these were purified and fed to rats. Beans contain considerable amounts of hemicelluloses which are not digestible but are easily fermented in the intestine with the formation of gas and frequently cause flatulence. The supplementary relations of pea and bean proteins for other proteins are discussed elsewhere (p. 116). There is little vitamin A in these seeds. Their content of vitamin B is similar to that of the cereal grains. Steenbock (1921) found that the vitamin A content of green peas having yellow pigment was higher than peas which did not contain yellow pigment.

A matter of great importance in comparing the relative values of the proteins of the legume seeds with those of meats, is the extent to which each supplies an abundance of those particular amino-acids which are not yielded in optimal amounts by the proteins of the cereal grains and tubers. The cereals form in temperate climates the greater fraction of our foods and supply most of the protein and energy of the diet. The milled products of these contain proteins of relatively low value and without exception are deficient in certain amino-acids. Combinations of these milled products mutually improve each other to a certain extent, but there are some essential amino-acids which are not abundant even in mixtures of several grains. When improving a dietary consisting largely of milled cereal products one of the important factors is the enhancement of the protein content. It is not so important to secure a high intake of protein as to have proteins of excellent quality. Relatively small additions of the right kind of protein greatly enhance the value of the proteins of cereals.

Soy Beans.—Soy beans have long been an important article of diet among the Chinese and Japanese peoples. Adolph (1922) mentions the great extent to which the soy bean enters into the diet of the Chinese people. He states that the Buddhist monks subsist largely upon soy bean curd. The soy bean has been

grown within recent years somewhat extensively in the United States. The studies of Daniels and Nichols (1917) and of Osborne and Mendel (1917) show that this seed belongs in the same class with peas and navy beans. Its proteins, which are adequate when fed at a plane corresponding to 17 per cent or more of the diet, can support growth when they form the sole protein supply. The vitamin content of soy beans is essentially like that of peas and navy beans. The legume seeds as a group are deficient in calcium, sodium and chlorin.

Peanut.—The peanut is a legume seed. It is used in the roasted state in America as a confection. Peanuts are grown extensively in the south for feeding hogs, but if fed too liberally they produce oily pork. To avoid this condition the animals are frequently finished on some other feed before being sent to slaughter.

Daniels and Loughlin (1918) conducted experiments with Spanish peanuts. Their results show that these have the dietary properties common to seeds and that they have no marked peculiarities. Their greatest deficiencies lie in their mineral content and in vitamin A. Johns and Jones (1917) have shown that the proteins of the peanut are usually rich in lysin.

Johns and Finks (1921) found tomato press seed cake to contain sufficient vitamin B for the rat. They found palm kernel meal when fed at 40 per cent of the diet not to furnish sufficient of the vitamin B for growth.

Cotton-seed Flour.—This product is prepared from the cotton-seed. In its manufacture the oil, hull and a considerable amount of resinous substances are removed. The flour has a nutty flavor and an effort has been made to promote it as a human food. Its dietary properties have been studied by Richardson and Green (1916), by Osborne and Mendel (1917), and by Wells and Ewing (1916). Like all other seeds, the proteins are complete when fed in the mixture in which they occur in the seed. The deficiencies of the product are those common to seeds, viz., lack of sufficient calcium, sodium, chlorin, and probably phosphorus and vitamin A. Osborne and Mendel (1917) found the proteins of this flour to supplement maize gluten, a commercial product obtained in the manufacture of corn starch. For this purpose they are equal to the proteins of brewer's grains, but inferior to lactalbumin.

Cotton-seed, and the press cake obtained in the manufacture of cotton-seed oil, contains a poisonous substance to which

Withers and Carruth (1915) gave the name *gossypol*. The oil extracted from the seed either by pressing or by extraction with fat solvents is also toxic to animals because of the presence of this substance. Carruth (1917) found that cooking the meal or heating it in the hot press method for extracting the oil causes a change which renders *gossypol* no longer soluble in oil or ether. It therefore remains in the meal. While cotton-seed flour or meal is a valuable feeding-stuff for animals, its use as a human food is not to be recommended because no assurance is yet offered that the toxicity of the products can be removed entirely. The same may be said of cotton-seed flour. Macy and Outhouse (1924) have given a resumé of the investigations of cotton-seed flour as human food.

Leaves.—The leaf has dietary properties very different from the seed. The contrast between the state of nutrition which can be secured in omnivorous animals with suitable mixtures of leaf and seed, as compared with complex mixtures of seeds, was first pointed out by McCollum, Simmonds and Pitz (1916-1917). It has not been found possible to secure satisfactory nutrition in animals fed exclusively seeds and seed products. The alfalfa leaf was first studied because the immature leaf is available as a commercial product known as alfalfa flour.

The dietary properties of mixtures of leaf and seed are well illustrated by a series of nutrition experiments carried out with mixtures of seed 60 per cent and alfalfa leaf 40 per cent. The seeds employed included wheat, maize, rolled oats, rice, pea and navy bean. The amount of growth with mixtures of one kind of seed with one kind of leaf is much greater than can be secured with such diets as a combination of wheat, maize, oat, hemp seed and millet seed in any proportions. A mixture of these seeds can support a fair amount of growth if its inorganic deficiencies are corrected, but without additions of mineral salts almost no growth can be secured. Chart VII shows typical growth records of animals confined to diets of leaf and seed. Among the seeds studied the oat kernel is best supplemented by the alfalfa leaf. A simple mixture of 60 per cent rolled oats and 40 per cent alfalfa leaf induces in the rat nearly normal growth. Animals grown on this diet have shown moderate fertility and fair success in the rearing of young. They were not nourished in the optimal manner, for they fell considerably below the maximum capacity of well-nourished animals in respect to fertility and rearing of young.

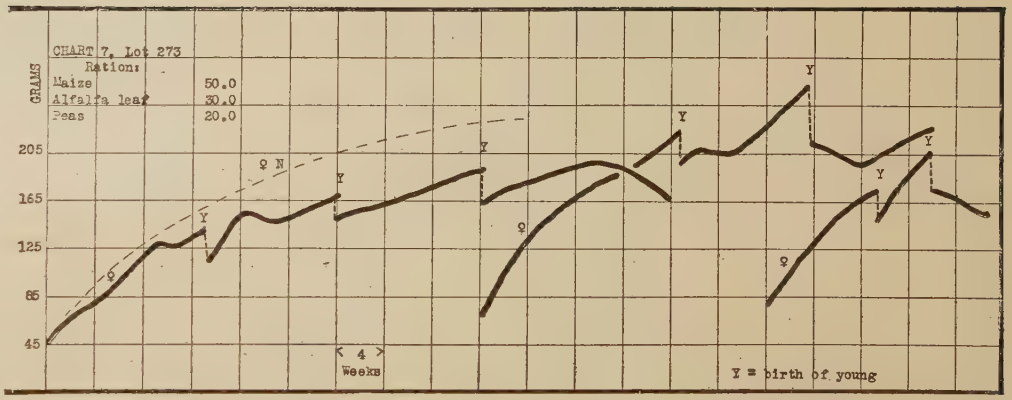


CHART VII.—This series of curves show the records of a mother, her daughter and granddaughter all of which were restricted throughout life to a diet consisting of maize, alfalfa leaves and peas. This simple mixture makes a fairly satisfactory vegetarian diet. This mother and her offspring were not so large as other animals in the same group, but her record is presented because young of the fourth generation, a great-great-granddaughter, was successfully weaned on this diet without any noticeable deterioration in the vigor of the family. The diet was not of the best quality, for the infant mortality was high, and there was a tendency for the animals to remain undersized. It shows, however, that it is easily possible to successfully rear rats on a strictly vegetarian diet.

An examination of the leaves of other plants showed that in a general way these can be classed together as foodstuffs of similar character. They resemble one another just as do the seeds in their nutritive properties. The dry leaf usually contains from two to five times as much ash as does the seed, and is always especially rich in just those elements in which the seed is poorest, viz., calcium, phosphorus, sodium and chlorin. It follows that the leaf supplements the inorganic deficiencies of the seed. The leaf contains in most cases much more of the vitamin A than does any seed. The protein of dry leaves varies from 8 per cent in such fleshy leaves as cabbage, to more than 15 per cent in alfalfa and clover, and to 30 per cent or more in the dry leaf of the turnip. Not all of the nitrogen in leaves is in the form of compounds which have a nutritive value. From the data available the proteins of the leaves appear to supplement the proteins of the seeds. The leaf supplements all the deficiencies of the seed, but not necessarily in a highly satisfactory manner in every case.

Differences in Function of Leaf and Seed.—It is interesting that the leaf of the plant shows such decided differences in nutritive properties as contrasted with the seed. The function of the two structures gives the clue to the reason. The seed is composed of a germ, which in most cases forms but a small part of the seed, together with a relatively large endosperm. The germ consists of living cells, which respire and which are capable of germination. In wheat the germ constitutes 1.5 per cent of the kernel. The endosperm contains large amounts of reserve materials such as protein, starch, sugars, fats and mineral salts. These are not living matter but its by-products. The endosperm is, therefore, in most respects comparable to a mixture of purified food substances. Experiments have abundantly demonstrated that the endosperm contains relatively little of the vitamin B, whereas the germ is rich in it. The vitamin A is likewise largely localized in the germ. The germ in most cases forms such a small fraction of the seed that the seed is relatively poor in this substance as compared with the leaf. The latter is in large measure made up of vegetable tissue containing all the protoplasmic structures necessary for metabolism. The chorophyl of the leaf enables it to utilize the energy of sunlight, and from the carbon dioxid absorbed from the air, together with water and mineral salts derived from the soil, it builds up protein,

starch, sugar and fat, which are used for growth, or for storage in the seed or tuber.

Thin Leaves More Complete Foods Than Thick Ones.—The dietary quality of the leaves differs to a considerable degree. Some are thin, cellular structures which dry easily. These have little reserve food material and relatively little inert tissue of a skeletal nature. Others, like cabbage, have become modified as storage organs, and contain reserve food comparable to that stored in the endosperm of the seed. The cabbage leaf also contains more than the average amount of cellulose and related substances which serve as supporting tissue. The dietary properties of such leaves are modified by these peculiarities. The cellular elements are diluted by the more inert tissues and reserve food substances in them. The less a leaf partakes of the function of a storage tissue, the more pronounced will be the leaf quality as a food. The fleshy leaves tend to have in some degree the dietary properties of the seed, and in this respect stand between the thin leaves and the seeds. In the case of the alfalfa leaf, which is a typical thin leaf of high nutritive value as a forage plant, the content of vitamin A has been stated by Steenbock to be greater than its content of vitamin B. Steenbock and Gross (1920) found that 5 per cent of immature alfalfa leaf as the sole source of vitamin A in the diet supported normal growth and allowed the rearing of some young. They found lettuce, spinach and chard to contain vitamin A in similar amounts but lettuce appeared to be somewhat poorer in this dietary essential than the other leaves studied.

Osborne and Mendel (1920) found alfalfa and clover leaves to surpass tomato, spinach, cabbage, turnip, carrot, beet root and potato in vitamin B content. All of these contain the vitamin A. The beet root did not equal the other roots. This is in harmony with the data of Steenbock and Gross (1919). One gram of dried clover or alfalfa per day fed to a rat for vitamin B gave better growth than the same diet with 16 c.c. of milk. Mendel (1921) estimated that a tenth of a gram of butter fat or a tenth of a gram of dried tomato fed daily as a source of vitamin A was sufficient to enable rats to grow to maturity and maintain them for 14 months. Osborne and Mendel (1922) reported studies of the comparative richness of several leaf vegetables in vitamin B. Asparagus, celery and lettuce were found to contain more vitamin B in terms of edible product than apples and pears, or the juice of grapes. They found that calculated as fresh vege-

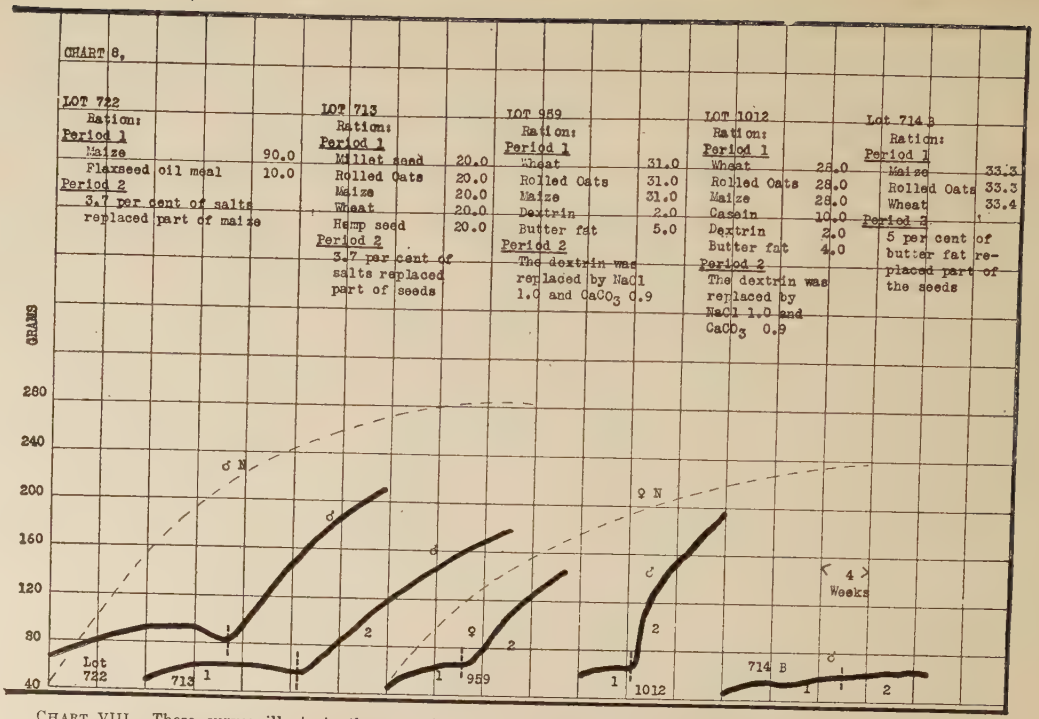


CHART VIII.—These curves illustrate the necessity of adding certain mineral salts, especially calcium salts and sodium chlorid, to mixtures of seed products. Lots 722, 713, 959 and 1012 were unable to grow in period 1, but in all cases growth was possible when salt mixtures were added. Lot 714 B made no response with growth when after a period of stunting butter fat was added to their diet. Mineral salts are the first limiting factor in preventing growth in animals fed seed mixtures. Compare with chart 7.

tables 2.2 gm. of asparagus, 11 gm. of lettuce, 10.8 to 17.2 gm. of celery, given daily as sources of vitamin B, produced excellent growth. Dandelion alone gave rather poor results, but 17.0 gm. of dandelion plus 0.2 gm. of dry yeast induced good growth.

Hume (1921) found white cabbage leaves inferior to green in vitamin A content. Steenbock and co-workers (1922) confirmed this observation. They also found that white sweet potatoes and white carrots contained very little vitamin A. The tops of white carrots were more pigmented with yellow and were richer in the vitamin than the roots. Green outside leaves of cabbage which did not fold upon the head contained more vitamin A than did inner white ones. Coward and Drummond (1921) state that etiolated seedlings and chlorophyl-free cabbage leaves do not synthesize vitamin A, whereas green leaves produce it. They also state that marine algae containing chlorophyl synthesize vitamin A, whereas organisms containing red pigments are not so active in this respect, and mushrooms devoid of pigment are almost completely lacking in vitamin A.

Tubers.—The tubers of certain plants constitute, after the seeds, one of the most important classes of energy-yielding foods. The white Irish potato is by far the most important of these in the temperate zones. In the Orient several kinds of tubers are widely used as human food. The dietary properties of the potato are what we should expect from its function as a storage organ for reserve food (McCollum, Simmonds and Parsons, 1918). The function of the potato is two-fold, reproduction of the species and a source of food supply for the young potato plantlet. The "eyes" of the potato represent groups of cells which are analogous to the germ of the seed. These are the places where the potato sprouts. Applemen (1918) believes that potato tubers contain substances necessary for the growth of shoots. He states that in planting the tuber it is important that it be cut into large pieces. He attributes the condition known as "spindling sprout disease" to a lack of this substance. There are within the tuber cells which are alive and which respire during its life. The interior of the potato consists almost entirely of water, starch, protein and mineral salts. It is analogous to the endosperm of the seed.

From the dietary standpoint the potato is to be classed with the seeds because it consists largely of reserve materials. The proteins of the potato and of the fleshy roots are not quite so valuable for the support of growth as are those of the cereal

grains when they serve as the sole source of this dietary factor. They are when raw an excellent anti-scorbutic food.

Rose and Cooper's experiments indicate that in human subjects the nitrogen of the potato is of extraordinary value for replacing that lost through daily catabolism in the adult (1917). McCollum, Simmonds and Parsons (1918) have studied this nitrogen by comparing with the protein of the cereal grains the value of the nitrogen of the potato. The experiments involved growth tests in the young rat and showed that potato nitrogen falls below the value for growth of that of cereal grains.

Fleshy Roots.—These are similar in a general way to the potato with respect to the relation between dietary properties and biological function. The roots we employ as foods are those which are highly modified as storage organs, and resemble the potato in possessing a high water and starch content and but little protein. In the case of the sugar beet the sugar in a great measure replaces starch. Steenbock and Gross (1919) demonstrated that the properties of the beet resemble those of the seed and tuber rather than those of the leaf. The dasheen and sweet potato have an inorganic content which resembles that of the seeds. An inspection of the analytical data relating to the composition of the ash of seeds, tubers and roots gives no promise that their inclusion in diets of seeds will correct the inorganic deficiencies of the latter. Feeding tests in which seeds and tubers or roots have been combined and supplemented with protein and butter fat (vitamin A), so that all the deficiencies of the mixture except the inorganic were made good, have confirmed this view. Denton and Kohman (1918 and 1920) studied the carrot by the biological method. Their results show that it was possible to maintain rats without growth for some weeks on a diet composed of carrots, sodium chlorid and a calcium salt. They found it necessary to add protein and vitamin A before the rat was able to grow. Morgan and Stevenson (1923) found that the French artichoke contains vitamins A and B, but less B than A.

Difference in Vitamin A Content of Roots.—Steenbock and Gross (1919) studied the more important fleshy roots, especially with respect to their content of vitamin A. Their results are of unusual interest for they showed that carrots and sweet potato, both of which contain much yellow pigment, are far better sources of vitamin A than are any of the roots which do not have a yellow color. Rutabaga, dasheen, red beet, parsnip,

potato, mangle and sugar beet appear from their results to contain practically none of this substance.

Fruits.—From the results of chemical analysis fruits appear to be comparable to the tuber and root vegetables. They all contain much water, and varying amounts of sugars, and are relatively insignificant as sources of protein or fat. They are more attractive to the palate than are most of the vegetables. They have mild laxative properties, and tend to increase elimination by virtue of their mild diuretic qualities. This is due in part to the extra water consumption, and also to the action of certain salts of organic acids.

Fruit juices or the edible parts of fruits differ from the cereal grains, and resemble the tuber and root vegetables in an important respect. There is an excess of basic radicles which renders their ash alkaline, whereas most seeds give an acide ash. Meats are also of acid character. The meat and bread portion of our diet, when oxidized in metabolism, leads to the formation of an excess of acid over base-forming substances. A certain alkali reserve in the blood is essential to maintain its capacity to carry carbon dioxid to the lungs for elimination. A common pathological condition, acidosis, exists when this reserve falls below normal. This is why the addition of fruits and vegetables to the diet tends to establish a proper acid-base equilibrium in the various fluids of the body.

The fruits differ from the leafy vegetables in containing little calcium. They resemble the tubers and roots in their mineral content. Osborne and Mendel (1919) found that 5 gm. per day of fresh apples or pears prevented beri-beri in rats. They also found that the edible parts of the orange, lime and grapefruit contain vitamin B in about the same amount as milk, and that they could be dried without losing their potency. Grape juice, apples and pears appear to be less potent. The tomato is richer than the others and has about half the potency of yeast when comparison is made on the basis of dry matter. They found (1922) that orange juice contains considerable amounts of vitamins A, B and C. Stammers (1924) found that 10 c.c. of orange juice daily did not contain sufficient vitamin B for satisfactory growth in rats but protected them against beri-beri. Morgan and Chaney (1924) found that grape fruit, fresh and concentrated, contains small amounts of vitamin A. The ether extract of grape fruit peel did not contain this vitamin. Orange juice,

peel and oil contained large amounts of vitamin A. Miller (1924) found fresh and canned pineapple to be good sources of vitamins A and B.

Dutcher and Outhouse (1923) found little if any vitamin A in any of several varieties of raisins. The vitamin B content differed in different varieties. Twenty to forty per cent of seedless raisins in the diet as a source of vitamin B sufficed to promote normal growth in rats. There was no vitamin C in market raisins.

Smith, Bergheim and Hawk (1921) report that strawberries are rich in vitamin C. Santos (1921) found that togi (sprouted mongo), okra, avocado, mongo, sweet potato leaves, and duhat (Eugenia jambolana) were relatively rich in B. Artichokes, bilimbi (Averrhoa carambola), and banana flour bud and bamboo sprouts were relatively poor in vitamin B. Mongo was found to be poor and togi when fresh relatively rich in C, but after cooking vitamin C had been destroyed. Santos also verified previous observations that in sprouting vitamin C is increased in mongo. Ten grams of mongo as a daily supplement to a scorbutic diet failed to protect guinea pigs from scurvy, while 5 gm. of fresh togi cured pigs of this disease. Shohl (1923) also found the Jerusalem artichoke very poor in vitamin B.

Fresh citrus fruits, *e.g.*, lemon, orange and lime, have long been known to prevent scurvy. Recent investigations have shown this property to be due to the presence of vitamin C. Byfield, Daniels and Loughlin (1920) found the anti-neuritic value of orange juice to be the same as that of an equal volume of milk.

Banana.—The banana has been studied as food for the rat by Benedict and Sugiura (1918-19), and as a nutrient substance for the banana fly by Loeb and Northrup (1917). The former found banana to be deficient in protein and in vitamin B when it constituted about 80 per cent of their experimental diet. Their studies verify the observation of Steenbock and Gross (1919) that yellow fruit contains more vitamin A than do fruits in general, or more than the roots and tubers not so pigmented. Benedict and Sugiura found that the banana supplemented with casein, and a source of vitamin B (yeast or carrot extract) proved inadequate for the production of milk by lactating rats. They state that the failure lay in the quality of the milk and not in the quantity, since the mothers failed to succeed with both

small and large litters. They suggested that a special vitamin is necessary for lactation, a view more recently expressed by Evans.

Loeb and Northrup (1917) found sterile banana inadequate for the nutrition of the banana fly, but banana cultured with yeast was satisfactory. The interpretation placed upon this observation is that the yeast cell produces synthetically some substance or substances, not present in the banana, but necessary for the nutrition of the fly. Acuña (1923) says that 5 gm. of papaya (Vigna sinensis), 10 gm. of banana, and 20 gm. of papaya (Carica papaya) were needed as a supplement to a basal ration to furnish vitamin B for normal growth in rats.

Nuts.—Nuts, with the exception of the chestnut, are rich in protein and fat, and poor in carbohydrates. The edible portions of several of the more highly prized nuts contain the following amounts of protein: Almond 21 per cent; beech-nut 22 per cent; brazil nut 17 per cent; chestnut 6.2 per cent; pecan 11 per cent; walnut 18.5 per cent. The fat content of these nuts is on an average: Almond 55 per cent; beech-nut 57 per cent; brazil nut 67 per cent, chestnut 6.2 per cent; pecan 71 per cent; walnut 64 per cent. The digestible carbohydrate of most nuts is very small. The chestnut is unique among the ordinary nuts in that it contains about 45 per cent of starch and little fat.

The flavors of nuts are especially appetizing and they are highly prized food-stuffs. They are the seeds of plants and one would predict that their dietary properties are such as to make them fail to supplement the cereals, legume seeds, tubers, roots or fruits, except in respect to the protein factor. A diet composed of such combinations of food may be balanced so far as chemical analysis can show, but be defective in other respects.

Cajori (1920) estimated that nuts had about the same value as a source of vitamin B as do the cereal grains. Drummond and co-workers (1920) examined the brazil nut, Barcelona, peanut, walnut, almond and butternut. Although containing a large amount of fat they all contained small amounts of vitamin A. This is interpreted as evidence that vitamin A is formed in the green parts of the plant. It is not stored to any appreciable extent in the seed or other resting tissue. Mignon (1923) found that cold pressed walnut oil when fed at 20 per cent of the diet did not contain sufficient vitamin A to prevent ophthalmia. She says that by extracting blanched almonds with 95 per cent hot

alcohol she prepared an extract which produced normal growth for 150 days when fed at 20 per cent of the diet. Her curves show subnormal growth. Morgan and co-workers (1923) found 20 per cent of almond oil did not cure ophthalmia in rats. Rose and MacLeod (1922) found rats to fail even with 15 per cent of almond oil as a source of the vitamin A. They believed the nut to be a fairly good source of the vitamin and that the failure was due to some interfering substance in it.

Animal Tissues.—Veal, beef, pork, lamb, fish and fowl are among the commonest animal products used as human food. Animal tissues must be differentiated into two groups, viz., glandular organs as the liver and the kidney, and the highly specialized tissue as the muscle. We are justified in saying that those organs which are the seat of metabolic activity of the type concerned with the elaboration of secretions or of intermediate or end products of metabolism are more complete foods than are the supporting and contractile tissues. It was predicted, in the light of the marked differences in the dietary properties of the leaves of plants as contrasted with the storage tissues that comparable differences would be found in the nutritive value of animal tissues having different functions. Experiment has shown this to be warranted.

Watson (1904 and 1906) restricted birds to an exclusive diet of muscle meat. He observed profound pathological changes, viz., nervous disturbance and paralysis (polyneuritis), hypertrophy of the thyroid and of the parathyroids. He also studied the effects on rats of an exclusive diet of horse and of beef muscle (1906) and found that young rats died and that the older ones were inferior in their ability to produce and rear young.

Vitamin Content of Animal Tissues.—Cooper (1912-1913) made a study of the relative values of food-stuffs for the prevention of polyneuritis in birds. The results were expressed in terms of grams of fresh substance per day necessary to prevent the disease. His values are as follows: Ox muscle, 20; ox cardiac muscle, 5; ox cerebrum, 6; ox cerebellum, 12; ox liver, 3; sheep cerebrum, 8-15; fish muscle, more than 10; egg yolk, 3; cow's milk, more than 35; cheese, more than 8. McCollum and Davis (1915) showed that an ether extract of pig kidney or of codfish testicles contained vitamin A. They showed that heart muscle contains less of this substance than kidney. These results for heart and kidney were verified by Osborne and Mendel (1917-1918).

The results with muscle tissues are in marked contrast to those obtained with the glandular organs. Osborne and Mendel (1917-1918) showed that beef muscle is very deficient in the vitamins A, B and C. Meat extracts were also deficient in these respects. Dutcher and co-workers (1919) found raw lean beef deficient in vitamin C. Damon (1922) found that commercial beef extract or peptone did not contain vitamin B. Osborne and Mendel (1917-18) also found liver to be a good source of vitamins A and B. They found the proteins of muscle, liver, kidney and brain to be complete as sources of amino-acids for the support of growth when each served as the sole source of protein in the diet. Fresh liver is rich in vitamin C. Parsons (1920) found fresh rat liver rich in vitamin C.

Hoagland (1923) states that lean pork is rich in vitamin B. He says it compares favorably with the vitamin content of liver and kidney. Beef, he says, contains less vitamin B than pork, and mutton stands between these. Heart is reported to be richer in B than liver or kidney. These results are not in harmony with those of others who have compared lean (muscle) meats with glandular organs. Wright (1923) found that fresh beef, mutton, lard and pork, as well as beef which had been stored two years, lamb stored three years and pork stored nine years, were all comparable in their content of vitamin B. He concludes that storage of meat is without effect on the vitamin B content.

Animal Fats.—McCollum and Davis (1913) and also Osborne and Mendel (1913) found that lard had no appreciable growth-promoting properties when added to a diet complete except for vitamin A. The latter found cod liver oil to be an excellent source of vitamin A. They examined (1915) the more liquid portion of beef fat and found it to contain this dietary factor, whereas the unfractionated fat was very poor in it. Halliburton and Drummond (1917) obtained similar results in their study of the nutritive properties of various kinds of margarines.

Daniels and Loughlin (1920) have presented data which indicate that some samples of lard contain demonstrable amounts of vitamin A. Drummond and co-workers (1920) believe that a pig can store vitamin A when receiving it in abundance. They bring out the interesting point that the concentration of vitamin A in pig fat, even in grass-fed animals, appears to be considerably lower than in the fat derived from grass-fed cattle, and much lower than that usually found in butter. Since pigs are

not ordinarily fed diets rich in this vitamin, lard is not a reliable source of it. Drummond (1921) confirms the view that the vitamin A content of beef fat like that of lard depends on the diet of the animal. Samples of hydrogenated fats of animal and vegetable origin and lard compound are usually devoid of appreciable amounts of vitamin A. Whale and other marine animal fats contain it but it is destroyed in the process of hardening. Osborne and Mendel (1921) found no essential difference in the results obtained with lard extracted with alcohol, commercial lard and lard rendered at comparatively low temperatures. Mallon and Clark (1922) found 30 per cent of lard from a pig fed a diet rich in vitamin A did not contain enough of this substance to prevent xerophthalmia. Rendering probably results in oxidation of a part of vitamin A.

Vegetable Oils.—Numerous experiments by various investigators have shown that without exception vegetable oils such as olive oil, cotton-seed oil, maize oil, soy bean oil, coconut oil, sesame oil, etc., are essentially without value as a source of vitamin A. On the other hand, a mixture of substances extracted by fat solvents from alfalfa leaves has been shown by Osborne and Mendel to contain some vitamin A. While fat solvents may extract small amounts of the vitamin, fats extracted from vitamin-rich foods by this means are not to be regarded as effective sources of vitamin A. Halliburton and Drummond (1917) tested coconut oil and nut butters and concluded that they are practically lacking in vitamin A. Jansen (1919) confirmed the observation of others that coconut oil contains very little, if any, vitamin A, and places it in the same category with olive oil.

Stammers (1924) reported experiments with two samples of maize oil, one crude and rancid, and one refined. His experiments indicate that the oil contained vitamin A in amounts comparable to that found in palm oil and also winter butter fat. He believes that the vitamin content of vegetable oils depends in great measure on the processes which they undergo during refinement. He confirms Drummond and Coward (1920) who also found that the vitamin A in vegetable oils depends upon the source from which the oil is derived and upon the method of preparation and refinement.

Fish Oils.—The livers of fish do not serve as a storehouse of carbohydrate (glycogen) as do the livers of mammals, but are loaded with fat when the fish is in prime condition. All

liver oils from fishes thus far studied have been shown to be excellent sources of vitamin A. This has also been demonstrated by Sekine (1920) for the oil from the liver of the basking shark and also for that from the abdominal muscles of tuna. He found the oil of the meat of Shya-chi-uo to contain liberal amounts of vitamin A.

Milk.—Milk is the most satisfactory single article of food which is consumed by man. It is not a complete food when taken over a long period as the sole source of nutriment. It is the one food for which there is no effective substitute.

Milk is a very effective food for supplementing the deficiencies of other substances such as cereal grains, tubers and fleshy roots (Lane-Clayton, 1916; McCollum, Simmonds and Parsons, 1921). It is rich in both calcium and phosphorus, and many vegetable foods are relatively poor in these elements. Calcium and phosphorus are prominent constituents of bone. There are but two classes of foods which are rich in calcium, the principal element in lime. These are milk of animals and the leafy vegetables. All other foods fall below the nutritive requirements of man and animals in calcium. The body is very sensitive to deviations from the optimal concentration of calcium in the food. Serious damage results to the bone structures if the diet is not properly constituted.

Dietary Properties of Milk.—Milk contains about 87 per cent of water and 13 per cent of dissolved substances consisting for the most part of proteins, milk sugar and fats. It is too bulky to be satisfactory as the sole food for the adult. Its value is as a supplement for other foods, correcting their deficiencies. The fact brought out by nutrition investigations is that most animal and vegetable foods are singly or collectively faulty in sufficient degree to interfere with the physical development of the young and to lead to early deterioration of the adult. A most important consideration to be kept in mind is, therefore, the selection of food so as to compensate for the deficiencies of one article by those of another and thus make a properly balanced dietary. For this purpose milk is the ideal food. Milk and the leafy vegetables are the only foods so constituted as to make good the deficiencies of cereal grains, legume seeds, tubers, roots and muscle meats. For this reason McCollum (1918) proposed that these two classes of foods should be designated "protective foods" in order to give them the prominence they deserve. Fresh fruits or certain raw vegetables should be in-

cluded in the diet regularly in order to insure a sufficient amount of the vitamin C.

The supplementary value of milk for other foods depends upon its unique calcium content, on the quality of its proteins, and on the quality of its fat.

It is of great interest that recent investigations have shown that the proteins of muscle, liver and kidney are more valuable for enhancing the values of cereal and legume seeds than are the proteins of milk. It is only in this respect, however, that the animal tissues surpass milk (McCollum, Simmonds and Parsons, 1921).

Great uncertainty exists as to the vitamin B content of milk, and considerable variation is known to exist in the content of A and C. Hopkins (1912) reported experiments in which 2 to 3 c.c. of milk to the basal diet of a young rat supplied it with sufficient of both vitamins A and B for growth response. On the other hand Osborne and Mendel (1918, 1920, 1922) found that 10 to 16 c.c. of milk are necessary to provide sufficient vitamin B for a rat whose diet is adequate in other respects. Johnston (1921) and Dutcher and co-workers (1920-1921) have reported results sustaining the contention of Osborne and Mendel that the small additions of milk made by Hopkins are inadequate to supply sufficient vitamin B for the rat. Hopkins (1920) reiterates the view, based on a repetition of his experiments, that 2 to 3 c.c. are sufficient.

McCollum, Simmonds and Pitz (1916) demonstrated the inability of little rats to grow when nursed by mothers whose diets were deficient in either vitamin A or B. They drew the conclusion that the lactating female cannot put the vitamins into her milk unless they are furnished by her food. Kennedy and Dutcher (1922) report that the diet of the cow determines the content of the vitamins A and B in her milk. Ten c.c. per day of summer or winter milk is adequate to furnish A and B for the rat provided the ration of the cow carries each in amounts adequate to meet her requirements. Typical winter farm milk was deficient in vitamins. These investigators assert that the effect of the vitamins is not necessarily one of appetite stimulation but rather a stimulation of metabolic processes which promote growth. Drummond and co-workers (1923) present data confirming the relationship between vitamin A content of the food and that of milk. The increase of the vitamin A content of the milk produced by feeding cod liver oil to cows was not

accompanied by a rise of lipochrome pigments as is the case when cows are fed on grass. Doses as large as 4 ounces per day of a good quality of oil produce no fleshy flavor in the milk or butter.

Reports of results of experiments where milk was the sole food of rats are very conflicting. The cause of this is not yet clear. Mattill and Conklin (1920) say when milk is supplemented with ferric citrate growth was much better than without it, but reproduction was not successful. Possibly milk is lacking in both quantity and quality in substances necessary for adolescent growth and reproduction. The growth of rats on this ration and also on 5 per cent of yeast was normal. Whether the yeast contained something unique remains to be seen.

Mattill (1921) found that on a diet of 93 per cent dried whole milk, 2 per cent salt mixture, and 5 per cent yeast, a fourth generation has been obtained in white mice. Animals on this food without yeast became pregnant and young were born but they were small and died within 3 to 4 days. Mattill (1922) fed rats solely upon whole milk powder. He observed failure of adolescent growth and reproductive ability in female rats. The defect was not corrected by diluting the milk powder with lard, starch and salts in varying proportions. Males grew normally but their reproductive efficiency was questionable. Additions of "protein-free milk," of cod liver oil, or of traces of potassium iodid after the animals had reached the age of 150 to 175 days did not result in fertility or renewed growth. Ordinary stock rat food also failed to restore them to normality. When animals that grew on the stock diet were transferred to a milk ration at about the age of adolescence, they likewise failed to rear their young or were sterile. The addition of a small amount of yeast to the milk ration caused the female to have young regularly and repeatedly, but none ever lived beyond a few days.

Sherman (1921) describes the results of feeding combinations of bread and milk, bread and apple, bread and turnip and milk and wheat. Sherman's data indicate that diets in which milk furnishes from one-third to two-thirds of the total solids were superior to diets similarly constituted but with smaller percentages of milk. Rose, MacLeod and Bisbey (1923) studied the maintenance value of milk, bread and milk, and meat and soy bean curd in human nutrition. Their basal diet contained arrowroot starch, dextrimaltose, lactose, butter fat and apple. Young women served as subjects in experiments covering 12 to 15 days.

In a 12 day period the nitrogen stored was 2.12 gm. with soy beans, 4.33 with meat, 41.43 with milk and 30.37 with bread and milk. Palmer and Kennedy (1923) say that it seems evident that milk as the sole source of food lacks something which is essential for the attainment of sexual maturity in the female rat. Mattill (1923) reports that Sherman has reared rats at least to the second generation on a milk diet.

Anderegg (1924) reports that on a diet of whole milk powder 70, iron citrate 0.2, agar 4, and dextrin 25.8 per cent, 9 female rats grew to maturity and gave birth to 70 young. Forty-seven died before being weaned. He secured 46 young in the third generation from a female confined to this food mixture. Fourth generation animals were weaned. Anderegg states that it is not necessary to assume, as Evans and Bishop have done, the existence of a new vitamin (X) for reproduction.

Sure (1924) secured growth with skim milk powder 50, gelatin 8, cystin 0.4 and ferric citrate 0.1 per cent, the remainder of the food mixture consisting of dextrin. The mortality of the young was high, but 3 young were weaned. He concludes that lack of fertility and of success with the rearing of young on milk diets must be attributed to factors other than proteins, the vitamins A, B or D. Sure (1924) found that the addition of a vitamin B extract to a milk diet did not increase fertility but enabled mothers to more successfully suckle their young. He confirms Evans and Bishop's observation that green leaves produce a profound effect on reproduction. He suggests calling the substance vitamin E which has been designated vitamin X by Evans and Bishop. Sure (1924) has found this substance to be present in the ethereal extracts of yellow corn, wheat embryo and hemp seed. He also found commercial cotton-seed oil and commercial olive oil to relieve sterility in female rats but this was not true for commercial coconut, linseed or sesame oils. McCandish (1924) found that milk cannot be used as the sole food for growing calves indefinitely without a stunting of growth. The deficiency of milk is not due to lack of vitamins A, B or C, but to a lack of roughage, a factor which he considers essential.

Fats of Milk Good Source of Vitamin A.—The fats of milk are the most important source in the American and European diet of vitamin A. This is not abundant in any seeds, tubers or roots, as compared with the amounts in butter fat of good quality. This is the anti-ophthalmic substance, vitamin A. It

is essential for growth or for maintenance. This will be considered more in detail later.

Several investigators have sought to determine the amount of vitamin A left in skim milk as compared with whole milk. The results would, of course, be greatly influenced by the completeness of removal of fat in skimming. Hart (personal communication) informs us that experiments made in his laboratory indicate that probably over 90 per cent of the vitamin A in whole milk is removed in the skimming process. Drummond and co-workers (1921) concluded that the diet of the cow is the chief factor in the variation of the vitamin A content of milk. They point out that the processes in butter making which expose it to air at high temperature may cause the destruction of vitamin A.

Smith (1922) found vitamins A and B to be present in typical samples of ice cream.

Ghose (1923) found that Indian ghee was as good a source of vitamin A as butter. Ghee adulterated with lard, mutton fat, coconut oil and almond oil was poor in the vitamin.

Widmark (1924) states that the export of butter from Denmark is the cause of the prevalence of ophthalmia. The population finds in margarine a substitute for butter. A few years ago margarine was prepared from animal fats which contained a little vitamin A, but during recent years there has been an increasing use of vegetable margarine, since this is cheaper. The latter is almost devoid of vitamin A.

Effect of Pasteurization on Vitamin, Calcium and Phosphorus Content.—Pasteurization as applied to milk is solely for the purpose of destroying certain disease-producing organisms which it may contain. Much interest has centered upon the possible destruction of vitamins in milk through heating it to 143 to 145° F for 30 minutes, as is done in the commercial process. All tests thus far reported support the view that milk loses its vitamin C content in great measure in pasteurization but that neither the vitamin A nor B is appreciably affected.

Daniels and Loughlin (1920) report experiments which show that in the process of pasteurization the calcium salts of milk are rendered more or less insoluble, depending on the length of time the milk is heated. In this insoluble form they may be lost through adhering to the container. This has long been known because of the formation in the pasteurization apparatus of a deposit called "milk stone." They emphasize that in the

case of long pasteurization or slow heating this is accentuated, and that considerable amounts may be separated from evaporated milk on standing. Their results show that where special care was taken to include the insoluble material by colloidal suspension in the evaporated milk, results comparable to those on raw and quickly boiled milk were obtained. Rats fed superheated milk with dicalcium phosphate addition made a normal gain. They believe that the inferior growth of rats on long-heated treated milk and superheated milk appears to be due wholly to the readjustments of the inorganic complexes, rendering assimilation difficult. Daniels and Stearns (1924) again emphasize that in heating milk phosphorus and calcium compounds are changed into a form which influences adversely their availability.

Milk Frequently Soured to Preserve It.—Milk is not only the most perfect human food but is likewise an unsurpassed nutrient medium for the growth of bacteria. Hence it is very liable to spoil through the growth of microorganisms unless it is properly handled. Pastoral peoples living under primitive conditions promptly sour all milk by permitting a vigorous growth of certain races of lactic acid-producing bacteria to grow in it. This protects it against proteolytic organisms which produce unwholesome decomposition products. The rise of acidity is so rapid that all other organisms and certain yeasts are killed or their growth inhibited. Sour milk is a wholesome food, and is used in enormous quantities in Asia, Arabia, the Balkan States, northern Africa and in the grazing sections of Abyssinia. Wherever it is used in liberal amounts as human food the people are of exceptional physical perfection. This will be discussed later.

Blackham (1920) has examined the milk supply in the tropics derived from the cow and the buffalo. The private milk supply is highly adulterated and largely contaminated. Buffalo milk contains about twice as much fat as does cow milk. Both in England and in India the bacterial content of milk is often greater than that of sewage. The solution of the milk problem in India is the development of some substitute for fresh milk such as dried milk powder which can be kept indefinitely in a dried state.

The number of bacteria in milk depends upon the temperature and age and the amount of dirt which contaminates it. In America it is the custom to market liquid milk either raw or

pasteurized. In order that this may be done in a satisfactory manner great care must be exercised in respect to cleanliness of milkers, of cows and of utensils. Refrigeration must be effective and delivery prompt. There has been steady progress in the supervision of the milk supply of cities in recent years and America has the most wholesome milk supply in the world. All city milk should be pasteurized. This is effected by heating the milk during 30 minutes to a temperature of 145°, followed by prompt cooling and bottling in sterile containers. This prevents the spread of typhoid fever, scarlet fever, septic sore throat, tuberculosis and other milk-borne diseases.

Bacteria in milk are related to infant mortality. Children fed clean milk with few bacteria have a lower death rate than those who take milk with high bacterial counts. Organisms which are relatively harmless to adults may cause diarrhea in infants and young children, or at least set up intestinal inflammation.

City Milk Should Be Rigidly Inspected.—The desirability of thorough control of a city milk supply through the agency of the health department has been fully justified by the results. Bacterial counts should be made regularly of market milk. They are an index to the care exercised in keeping healthy cows and in exercising cleanliness on the farm, refrigeration and promptness of delivery; also as a basis of condemning milk which is unwholesome because containing dirt, filthy or decomposed materials. Dairy inspection has come to be a well-recognized method of insuring a wholesome milk supply.

Eggs.—Eggs contain everything that is necessary for the growth and maintenance of the body, but are poor in calcium, and are unbalanced with respect to certain other food principles. Eggs are very rich in fats and related substances, and are nearly lacking in carbohydrates. They favor putrefactive processes in the alimentary tract.

McCollum and Davis (1913) showed that the fats of eggs are fully comparable with butter fat as a source of vitamin A. The proteins of egg are believed to be of high biological value. In the developing chick there is considerable absorption of lime from the shell, and in this way the shortage of calcium in the organic content of the egg is made good. When we eat eggs we remove the shell and thus eat the part which is deficient in lime.

Osborne and Mendel (1923) found egg yolk to be poor in

vitamin B. One-half gram of fresh egg yolk per day was necessary for growth in a 100 gm. rat. They estimate that a quart of whole milk is equivalent to 6 or 7 whole eggs in vitamin B. The high content of vitamin B stored in seeds and in eggs of birds and fishes suggests a due provision for the needs of the young during early life.

It will be seen that eggs may have an important place in enhancing the diet. They should be eaten with a diet rich in carbohydrates to discourage the tendency to putrefaction in the intestine. They should be used to confer palatability on other foods rather than as a prominent constituent of the food supply. They have, however, dietary properties which make them desirable to use when the item of expense is not prohibitive.

The Carbohydrates.—Cane sugar, beet sugar, starch, glucose, syrup, honey and candy are sources of carbohydrates. They are essentially of value as sources of energy. They differ to some extent in the rate at which they are absorbed, the simpler sugars being more readily digested and absorbed than starch. These are all lacking in vitamins of every description with the possible exception of honey. Dutcher (1918) concluded that honey contains a small but negligible amount of vitamin B. Faber (1920) did not find honey to protect guinea pigs from scurvy. Hawk, Smith and Bergheim (1920) found that the amount of vitamins A, B and C in strained honey was negligible. Comb honey was reported to contain vitamin A. Scheunert (1923) fed honey from 3 sources and found it lacking in vitamins A, B and C.

Aeptler (1922) made feeding experiments with royal jelly and found it lacked sufficient vitamin A to promote growth in young rats. It is suggested that the queen larva has but a small requirement of vitamin A. Royal jelly was relatively rich in vitamin B. This is apparently derived from pollen, since Hawk and his co-workers (1921) have shown that honey contains very little vitamin B.

Sea Foods.—Foods of the class to which oysters, crabs and clams belong assume a new importance in the light of the nutrition studies which have so clearly shown the inferiority of muscle meats as compared with the secretory organs and tissues which are concerned with the transformation of foods to end-products of metabolism, *i.e.*, the glandular organs. In clams, oysters and soft crabs, we eat the entire animal except the shell. Their consumption insures that all the uncharacterized food substances

will be present in the diet, and makes these supplementary in the sense that they are likely to add to the diet some principles which it contains in amounts below the optimum. Sea foods are of special significance in that their iodin content is higher than that of land plants or of the flesh of land animals.

Randoin (1923) detected vitamin C in raw oysters by experiments on guinea pigs. The shellfish doubtless played a most important rôle in the nutrition of early man. Numerous shell-heaps or "kitchen middens" of enormous proportions occur on the Atlantic coasts of America and Europe. These were the sites of human habitations over long periods and indicate that shore dwellers depended in great measure on shellfish for their sustenance. Their type of diet represented a peculiar kind of carnivorous feeding which was very satisfactory.

The demonstration in recent years of the unique quality of fish oils as a source of the vitamin D suggests the importance of fish in the diet, which was hitherto unsuspected. The percentage of fat in the different species of fish varies greatly. Salmon, shad, herring, butterfish and mackerel are well-known fatty fish, but cod, haddock, cusk, pollack and flounder are lean fish. The fatness of fish often varies greatly with the seasons. Salmon and shad, which are very fat before spawning, become very lean immediately afterwards (Tressler, 1923). Drummond (1918-19) has studied the value of fish proteins in the diet, and finds them essentially the equivalent of lean meats from mammals.

An examination of this chapter reveals the fact that there are but two classes of calcium-rich foods—milk of animals and the leaves of plants. It has been shown that foods which have the same functions have, in a general way, the same dietary properties. Thus the tuber and root vegetables are more or less similar in their nutrient properties, as respects inorganic elements, vitamins and energy values. Their protein content is low. All cereal grains are comparable in their dietary qualities, but differ in minor degrees. The special value of fruits lies in their suitability in most cases for providing raw food for its antiscorbutic value. With the dietary properties of the different food-stuffs understood as they are at present it should be easily possible to make combinations which will provide highly satisfactory diets.

CHAPTER X

THE VEGETARIAN DIET

Vegetarianism Generally Practiced as Dietary Fad.—Dietetic theorists have often advocated the adoption of a fleshless diet. The proponents of this system have broken up into schools the adherents of which differ in the fervor with which they espouse their cause. The most extreme of these are the enthusiasts of the fruit and nut diet and have been studied by Jaffa (1901-02). They experience esthetic pleasure in the thought that their foods grow and ripen away from contact with the soil and suggest, therefore, the purity and fragrance of orchard and of forest, the poetic joy of summer and the inspiration of the rural landscape. A second group adopts the vegetarian diet because of an abhorrence of the idea of taking animal life for the sake of food. These two classes of vegetarians regard flesh eating as unwholesome as well as unethical, and defend the view with a number of arguments that meat eating tends to moral debasement and physical degeneration. The third group, the lacto-ovo-vegetarians, who permit the use of milk and eggs along with a diet otherwise vegetarian, is less extreme in its ardor for ethical considerations, and adheres to its tenets because of conviction that it makes for physical efficiency in a greater degree than does the consumption of meat.

Faddists Discredit Themselves by Their Philosophy.—While some of the arguments offered in sober earnestness in support of abstinence from flesh foods are suggestive of mental invalidism, others demand serious consideration and an answer based upon scientific understanding. In the former category we may place the assertion that the anthropoid apes, regarded as cognates of our ancestors, live on fruits, nuts and cereals, and that this fact proves that such a diet is especially suited to the nutrition of man. One may well argue that if a change to meat eating in the era of the cave man has enabled him to develop mentally into what man is today, this change in dietary habits may have been indeed a great piece of good fortune. The low development of vegetable-eating animals weakens the force of

the argument. It may be suggested that it is no more advisable to permit the apes to be our guides in dietetics than in certain other ways of living. It is, however, well known that the apes eat insects, worms, eggs, small birds and such other animals as they are able to capture, and also the leaves of certain plants, as well as nuts and fruits.

There is little comfort for the vegetarian enthusiast in the comparison of the anatomical structure of the alimentary tract of man with that of animals whose diets are of different types. The dentition, the size and shape of the stomach, intestine and cecum of man clearly indicate suitability for a mixed diet. To the esthetic argument the meat eater may reply that he includes sufficient fruits, nuts and cereals in his diet to permit him to be inspired by the beauties of Nature. To the statement that a refined person cannot eat meat until it is cooked, and its flavor changed so as to disguise its origin, the reply may well be made that many fruits and vegetables are not at all appetizing until cooked and so flavored as to increase their palatability. The argument which was at one time considered to be irrefutable and to be of sufficient force to condemn meat eating rested on the content of uric acid forming substances (purins) in flesh foods. Chemical studies have shown that there are very nearly comparable amounts of purins in many vegetable foods, such as lentils, oatmeal, beans, asparagus, and notably in tea and in coffee.

Meat Eating Does Not Deaden Moral Faculties.—A stock argument of radical vegetarians is that meat eating deadens the moral and intellectual faculties. One who reflects that Moses, David, Solomon and Jesus ate flesh foods cannot be deeply impressed by it. Likewise the statement that animals living on a vegetarian diet are strong and tractable, while carnivorous animals are ferocious does not stand inquiry. The average bull or belligerent billy goat can strike terror into the heart of man with less provocation than is necessary to render dangerous a lion or a wolf. The gentle and happy disposition of the Eskimo on his carnivorous diet has frequently been commented upon.

A Valid Argument Against Meat Eating.—The more valid arguments concerning the use of meat as contrasted with the fleshless dietary regimen are based upon the view that meat is unwholesome and that it contains waste products having poisonous properties which tend to damage the tissues. This view is

upheld by experimental results. Professor Fisher (1907) of Yale conducted experiments with flesh abstainers and flesh eaters, and found the former possessed of greater endurance than the latter. The tests involved holding the arms outstretched, knee bending and other exercises. The flesh abstainers were three to six times as capable of endurance as were the flesh eaters. Similar results have been reported by Kellogg (1907 and 1909).

It would be unprofitable to review the old debate concerning the relative merits of meat eating and meat abstinence. There was no evidence of a conclusive nature upon which the question could be decided. Those not convinced by the endurance tests could argue that the vegetarians were enthusiastic exponents of a fad which they exerted themselves to the utmost to defend, whereas the meat eaters were not so determined to justify their practice. There were factors not then appreciated which are of vital importance in the discussion of the vegetarian diet. These can best be made clear by describing certain experimental work which has a direct bearing on the subject.

Slonaker's Study of Vegetarian Diet.—The most elaborate attempt to test the relative merits of a strictly vegetarian diet as contrasted with an omnivorous type of diet was made by Slonaker (1912). He fed a group of rats 23 vegetable foods, allowing free choice within limits. For comparison a similar group was fed the same foods of vegetable origin supplemented with moderate quantities of animal food. Several foods, raw or prepared, were offered at a time. No record of food consumption or of the amounts of the different foods eaten was kept. Accordingly the results cannot be used for critical examination except in a limited way. They are, however, of interest in showing how far instinct fails to guide an animal in the selection of food. Slonaker's list of foods included nearly everything which a vegetarian in southern California would be likely to have on his table, including seeds, the milling products of seeds, leafy vegetables, fruits, tubers and fleshy roots.

The vegetarian rats grew fairly well for a time, but became stunted when they reached a weight of about 60 per cent of the adult size. They never increased in weight beyond this point. The omnivorous controls grew to be of normal adult size. The vegetarians lived on the average 555 days, whereas the omnivora averaged 1,020 days. The vegetarians grew to be about half as large and lived half as long as did their fellows receiving animal food. Slonaker concluded that a strictly vegetarian diet was not

suitable for the nutrition of an omnivorous animal. He was not able to interpret the cause of the failure.

Even with Wide Variety Vegetarianism Leads to Disaster. —The results of Slonaker's experiments were published in 1912. At this time McCollum and Davis were securing the experimental data which revealed the differences in the growth-promoting power of different fats which established the existence of a hitherto unsuspected dietary essential. It seemed to them that the most probable explanation of the results of Slonaker was the absence or shortage in his vegetarian diet of the essential furnished so abundantly by certain animal fats, which later came to be designated as vitamin A. A low protein intake was suggestive of another cause for Slonaker's failure. With these ideas in mind they tried in 1914 an experiment similar to that of Slonaker, but so modified as to give the animals a much higher protein intake than his animals probably took, and an added supply of vitamin A.

McCollum and Davis fed their rats a diet which afforded a choice among the following list of foods: wheat, maize, rye, oat (rolled), wheat germ, maize gluten, wheat gluten, flaxseed oil meal, green clover, green alfalfa leaves, onions, peanuts, and cooked navy beans and peas. With the exception of the last two articles the foods were fed raw. It will be observed that in this list there are several vegetable foods having unusually high protein content. Maize gluten, a by-product of starch manufacture, contains about 25 per cent; wheat gluten, prepared by washing ground wheat free from starch, about 85 per cent; flaxseed oil meal 30 per cent, and wheat germ 30-35 per cent of protein. Animals are known to grow well on many diets containing 15 to 18 per cent of protein. It seemed that with this variety of protein to select from one possible cause of failure in Slonaker's experiments, viz., too low a protein intake, would be avoided. McCollum and Davis had at that time not discovered that the leaf of the plant is a moderately good source of the dietary essential vitamin A. It was assumed that when leaves and so many different kinds of seeds and seed products were supplied, there could be little doubt that everything a herbivorous animal requires was present in the food.

The rats fed this wide variety of vegetable foods, and supplied with liberal amounts of protein, duplicated in all respects the results of Slonaker. They grew at about half the normal rate for the first few weeks, then became permanently stunted

The addition of butter fat to the diet of some of these animals failed to benefit them in a noticeable degree. The answer to the question as to why rats did not thrive on such strictly vegetarian food mixtures was not secured from these experiments. It was, however, soon learned wherein lay the cause of failure.

Successful Nutrition on Strictly Vegetarian Diet.—McCollum, Simmonds and Pitz (1916 and 1917) fed a group of rats on a monotonous diet consisting of maize 50, alfalfa leaf (dry) 30, and cooked peas, subsequently dried, 20 per cent. The ingredients were ground together so that these proportions were eaten. The diet induced growth at approximately the normal rate, reproduction and rearing of a considerable number of young. The young grew to adult size and were successful in rearing a considerable number of offspring (see Chart VII). Without tasting, after weaning, anything other than this monotonous mixture, this family of rats remained approximately normal in vigor and successfully weaned the young of the fourth generation with no apparent diminution in vitality. At this point the experiment was discontinued.

Their next series of experiments (1917) was with diets derived solely from a mixture of a leaf and a seed ground together so that the two parts of the ration could not be picked out and separately eaten. In marked contrast to the failure of animals to grow on any mixture of seeds, it was found that a mixture of a seed and a leaf formed a diet on which considerable growth could be secured. Polished rice, which requires supplementing with respect to four dietary factors, viz., mineral salts, vitamins A and B, and protein, before it becomes complete for the rat, was found to induce growth when fed with leaves in the proportion of 60 per cent of seed to 40 per cent of leaf. On this simple and monotonous mixture rats grew from weaning time to 83 per cent of the adult size. One female produced two litters, but allowed them to die. A mixture of rolled oats 60 per cent and alfalfa leaves 40 per cent makes a better ration. On this mixture rats were observed to grow to the adult size and to reproduce and rear young. One female reared 14 out of 17 young in 3 litters. Maize and alfalfa leaf or wheat and alfalfa leaf were not so satisfactory for growth as the corresponding mixture containing rolled oats. Mixtures of the alfalfa leaf with the legume seeds, peas and beans gave still poorer results. The peas and beans were always cooked and subsequently dried before feeding.

A point of interest which it is well to emphasize, is that the bones of these vegetarian rats were of very good quality. There was no evidence of rickets in any of the young. This disease invariably develops in young animals restricted to a diet of cereal grains. The nature of the defects in the diet which cause this disease will be considered later. It is sufficient to say that a proper adjustment of the mineral elements in the foods, and the presence of an organic factor which exerts an important influence on bone formation, are indispensable conditions of normal skeletal development. The results of this experiment demonstrate that the mineral content of the leaf component of the vegetarian diet supplemented the inorganic nutrients of the seed portion, so as to favor normal bone development. It is also evident that this diet contained sufficient of the organic factor concerned with the metabolism of bone, since no skeletal defects were observed. In China and other parts of the Orient where green, leafy vegetables constitute a very important part of the food supply, rickets is almost unknown as a disease of children, whereas it is common in Europe and America, where the tendency in numerous homes is to derive a large part of the food supply from milled cereals, meats, legume seeds and tubers.

These experiences made it evident that there is nothing in vegetarianism *per se* which makes it impossible for an animal of the omnivorous type to be satisfactorily nourished on this kind of diet. It is only necessary to make the proper selection of food-stuffs, and to combine them in the right proportions. In the experiments described, in which the diet was made up of so simple a mixture as a leaf and a seed, optimum growth, reproduction and rearing of young were in no case secured. McCollum and Simmonds (unpublished data) have the results of a very extensive series of feeding trials in which the rations were made up of one seed, one leaf and one legume (pea, bean) in various proportions. These have failed to reveal any mixture which is quite the equal of that used in the first experiment, viz., maize 50 per cent, alfalfa leaf 30 per cent, and peas 20 per cent. It is possible that better mixtures of vegetable foods may be found but these results show definitely that for the omnivorous type of animal, whose digestive tract is small, the consumption of sufficient volumes of leafy foods is not possible. It is by no means a simple matter to derive the diet entirely from the vegetable foods and secure the optimum of well-being. The experi-

FIG 7.—This photograph shows the degree of success which has been attained in securing growth, reproduction and rearing of young in an omnivorous animal, the rat, with a diet of strictly vegetable origin. The diet is described on page 165. The data available seem to demonstrate that successful diets of plant origin can be secured only when the leaf of the plant is a prominent constituent of the diet. Those parts of the plant whose functions are those of storage organs (seeds, tubers and roots), do not serve as adequate diets, even when there is a wide variety in the food supply. The leafy structures are so constituted as to correct the deficiencies of mixtures of seeds, tubers and roots.

The albino and her daughter are shown above, and her granddaughter below. Young in the fourth generation were successfully reared, with no apparent diminution in vitality.

ments described demonstrate that wide variety may be of little value as a safeguard of nutrition.

Cause of Failure of Slonaker's Rats.—There are several reasons why Slonaker's rats did not thrive on the vegetarian diet fed them. It was low in protein. The leaves, which formed the only components of the food supply containing enough mineral elements to support growth, were fed in the fresh condition. In this form the water content and bulk are so great that it would be impossible for an animal whose digestive apparatus is no more capacious than that of an omnivorous rat to eat a sufficient amount of leaf to correct the inorganic deficiencies of the rest of the mixture. Physical limitations likewise led to failure to secure enough of the vitamin A. This would not form so important a fault as the inorganic deficiencies but would be an important depressing factor. Success would also turn upon the ability of the animals to instinctively select the best proportions of the food-stuffs offered them. The appetite is by no means a safe guide to the selection of foods.

Evvard's Studies on the Appetite as a Guide to the Selection of Food.—Evvard conducted experiments to study the extent to which the appetite and instincts of the hog aid in the selection of food (1915). As a rule in all these trials the animals were given a choice of only three foods, a cereal grain, a protein-rich food, and a plant leaf. The data secured seemed to show that there is some basis for the belief that this element of selection by the animal itself is worth taking advantage of. The value of the leaf in the food supply of the growing pig could not have been explained before the studies of McCollum and his associates with simplified diets and with diets restricted to a single food-stuff. Feeding such diets with single and multiple food additions led to a knowledge of the exact nature of the dietary faults of each.

Vegetarianism Viewed from Wrong Angle.—It will be clear from the discussion in this chapter that the vegetarian diet has been viewed from the wrong perspective. It is possible to make a fairly satisfactory diet of foods derived entirely from vegetable sources, but it is not easy to do so. It would be difficult for man to eat enough leafy foods to enable him to succeed with a strictly vegetarian diet. Such a diet will generally be of low protein content. The addition of small amounts of meat will be valuable when such a regimen is adhered to. Eggs, because of their relative richness in vitamins A and B, will be often

more effective supplements and the same may be said of small amounts of glandular organs.

Many of the people of China and of Japan because of poverty and overcrowding can afford but small additions of fish, poultry, eggs, or the flesh of mammals to their diet consisting in great measure of vegetable foods. The children are schooled from an early age to the consumption of leafy vegetables. Many weeds are eagerly sought after, and trees are stripped of their buds in spring to augment the supply of spinach, cabbage and other less leafy foods. An alfalfa field seeded by the Rev. Mr. Holding (personal communication) in north central China was eagerly appropriated for human food, although it had been intended to serve as a forage for animals. Millions of people in Asia have learned the unique nutritive value of green plants, which we in America have never learned to appreciate.

Carotinemia.—Miura (1917) reported that orange yellow pigmentation of the skin, which has been described under the term "aurantiasis cutis" by Beatz, is due to coloring of the subcutaneous adipose tissue by carotin present in mikan, squash, laver, etc. Owing to the affinity of fats for carotinoids he noted that the term carotinosis is more nearly exact than the term carotinemia. This excessive yellow pigmentation has since been repeatedly observed, especially among persons who eat too constantly of carrots, squash, and other yellow pigmented vegetables. Hess and Meyers (1919) reported carotinemia in hospital infants receiving carrots in their diet. On leaving carrots out of the diet the pigmentation disappeared. Hashimoto (1922) says pigmentation of the skin has long been observed among vegetarians in Japan, where Citrous nobilis or unusual amounts of squash are used as a partial substitute for rice among farmers. The condition simulates jaundice but differs from it in that the palms and the soles are more deeply pigmented than the rest of the skin, and the whites of the eyes are not colored as in jaundice. Palmer and Kempster (1919) made a detailed study of the carotinoids in the diet in relation to the color of egg yolks, and the ear lobes, shanks, etc., of birds.

CHAPTER XI

THE DIETARY DEFICIENCY DISEASES

SCURVY

There has long been a belief among both medical and non-medical observers that certain diseased conditions were the result of the continued use of a faulty diet. Of these scurvy was the one most definitely attributed to unsatisfactory food. Scurvy occurred in the thirteenth century among the armies of the Crusaders. After the discovery of America long voyages became more common and scurvy began to take a heavy toll of life among mariners. It became known as "the calamity of sailors" because of its frequency on shipboard. A notable instance was the voyage of Jacques Cartier in 1536. While in the St. Lawrence River he lost from scurvy twenty-six of his party, and saved the remainder by the use of an infusion of pine needles (Hirsch, 1885). In 1747 James Lind, a surgeon in the British navy, made experimental observations on sailors suffering from scurvy. By appropriate control experiments he showed that the medical treatment in vogue was futile, if not actually harmful, and that orange and lemon juices were a specific cure. He studied the antiscorbutic value of various fruits and green vegetables but was unable to determine that any one was superior to another. Lind confirmed the observations of Kramer made at the beginning of the eighteenth century during the war between the Turks and the Holy Roman Empire that dried vegetables would not prevent scurvy. A friend of Kramer asserted "that no moisture whatever could restore the natural juices of the plant lost through evaporation," because these were "altered by a fermentation which they underwent in drying." Lind observed the beneficial effects of cow's milk in the treatment of scurvy. This he explained on the supposition of milk "being a truly vegetable liquor, an emulsion prepared from the most succulent wholesome herbs." Lind recommended that lemon juice concentrated to a syrup by evaporation should be

carried in all ships and served to the sailors. The efficacy of fresh fruits and vegetables as remedial agents in the prevention and cure of scurvy was discovered by common experience. It became sufficiently established so that in 1795 the British navy began the regular administration of lime juice to the sailors. In 1865 the British Board of Trade adopted with equally beneficial results a similar regulation. Scurvy continued to be a menace to armies, appearing even during the World War because of a lack of knowledge among army officers of the dietary properties of foods.

Stevenson (1920) has given a very interesting report of an outbreak of scurvy in N. Russia, together with an experiment in test dieting during the World War. It appears that when there is absolute deprivation of fresh food scurvy may appear after about 6 weeks. The presence in the food of even very small amounts of articles which provide the vitamin C will defer the onset of the disease so that it may be months before it develops.

Professor Hopkins (quoted by Dupain, 1920) says he was very much impressed by what happened at a large preparatory school in England. During a winter term at this school the conduct of the boys grew vaguely unsatisfactory. The standard of work and play fell much below normal, the boys became listless and irritable and various forms of minor complaints were reported. The explanations forthcoming were insufficient. Throats were examined, drains flushed, and other hygienic measures were instituted, but nothing came to light, and the conditions grew worse. It was suggested that the diet be inspected. This seemed all right. The boys were well fed. However, it was found out that the diet supplied nothing in the way of uncooked foods and practically no greens. It was noted also that the little shop where the boys purchased fruit with their pocket money was closed. Upon a liberal introduction of fresh fruit into the dietary the whole trouble disappeared. The school had been suffering from incipient scurvy, due to lack of vitamin C.

Symptoms of Scurvy.—Lind, in discussing scurvy in sailors, made an observation which deserves our careful consideration to-day in relation to the effect which the diet may exercise on general well-being and behavior. He says "an uncommon degree of sloth and laziness which constantly accompanies this evil (scurvy) is often mistaken for the willful effect of the patient's disposition. This may prove fatal to many, some of whom when

obliged by their officers to climb up the shrouds have been seen to expire and fall down from the top of the mast."

Scurvy develops gradually. The adult loses weight, is anemic, pale, weak and short of breath. The gums become swollen, bleed easily, and frequently ulcerate. The teeth loosen and may drop out. Necrotic areas in the jawbone may result. Hemorrhages into the mucous membrane and the skin are characteristic. In the skin large blue-black spots develop after trivial injury, or even spontaneously. The ankles become edematous, and in severe cases there develops a hard, boardlike condition of the skin and subcutaneous tissues. Nervous symptoms of various types are seen. In the later stages of the disease, headache, convulsions and delirium appear.

Lime Juice Not Always Protective Against Scurvy.—Although the experience of the British navy and of several Arctic expeditions had convinced many persons that lime juice was a specific remedy for scurvy, there were a number of widely known outbreaks of the disease which tended to discredit the view that scurvy was due to the lack of some essential substance in the diet. One of these was the experience of Sir George Nares, who in 1875-77 made an expedition to the polar regions in the *Alert* and the *Discovery*. Both crews suffered severely from scurvy, although lime juice was regularly rationed to all. It appears that the lime juice was inferior, and perhaps adulterated. The history of the early events in the use of anti-scorbutic foods has been interestingly written by Alice Henderson Smith (1919). She states that what we now call the lemon was frequently called lime. The true lime is very inferior to the lemon in its antiscorbutic property.

In 1895-96 the British Army garrisoned at Chitral, India, suffered severely from scurvy, notwithstanding the fact that lime juice was administered daily. In 1902, the men who constituted the party of Captain Scott in his voyage to the Antarctic developed scurvy in spite of the fact that they were well supplied with West Indian lime juice. On this occasion the men were saved by the substitution of fresh seal meat for canned meats; mustard and cress were grown for consumption in the raw state. Probably the lime juice used in those cases where there was no protection against scurvy was adulterated, or had been heated to preserve it. It is now known that heating (contact with air) tends to destroy the substance which gives fresh fruits and vegetables their protective value.

The scurvy outbreaks despite the use of lime juice aroused in the minds of many the suspicion that scurvy might not be due to lack of freshness in the food. A number of theories were advanced to explain the etiology of the disease. Harley and Jackson (1900) accepted the view advanced by Torup of Christiania, that scurvy resulted from eating decomposed meat, and was a kind of "ptomain" poisoning. This view seemed plausible. When the disease was common the sailors were restricted essentially to a diet of salt meat and hard tack. The experiences of men in the Arctic regions with fresh and stale meats confirmed the supposition that the latter were inferior to the former. Stefánsson (1918) concluded that a factor of importance was the consumption of large amounts of salts with salted meats. He denied salt to his men as a protective measure against scurvy.

Scurvy Attributed to Acidity of Diet.—Wright (1895) compared the symptoms of scurvy with those induced by large doses of mineral acids, and from their similarity concluded that scurvy was due to acid intoxication. He suggested that a diet of cereals or of meat produced scurvy because they contain an excess of mineral elements which in metabolism are converted into acids. The following table gives a list of foods, with their relative yields of acid and base radicals, presented by Wright in support of his view. These afforded strong evidence that his views were correct. The values represent the oxalic acid equivalent of 100 grams.

TABLE XIV

Acid Food-stuffs	*gm.*	*Alkaline Food-stuffs*	*gm.*
Oats	1.69	Carrot	0.61
Barley	1.19	Turnip	0.32
Beef	0.27	Potato	0.27
Wheat	0.25	Onion	0.25
Egg	0.20	Cow's milk	0.17
Rice	0.10	Ox blood	0.13
Maize	0.07	Peas	0.13
		Lemon juice	0.12
Neutral Food-stuffs		Orange juice	0.12
Sugar		Beans	0.07
Fats		Sheep blood	0.07

It should be mentioned that the presence of organic acids in certain of the vegetable foods in the alkaline list is of no significance in this connection because these are destroyed by oxidation in the body.

It will be observed that those foods which were reputed to prevent or cure scurvy fall in the alkaline group. Wright believed that it was not essential for the diet to contain uncooked succulent foods but that it should supply sufficient alkaline radicals to make the diet somewhat basic.

Classic Experiments of Holst and Frölich.—Modern knowledge of the etiology of scurvy dates from the discovery by Holst and Frölich of the University of Christiania (1912) that guinea pigs readily develop the disease when confined to a diet of cereals or of bread. They pointed out that the syndrome thus produced was the analogue of scurvy in man. It was characterized by loosening of the teeth, inflammation of the gums, hemorrhages in the joints, decalcification of the epiphyses of the long bones, and the typical changes in the bone marrow. They observed that exclusive feeding of guinea pigs with carrots, turnips or dandelion did not lead to this condition, although animals on these foods suffered comparable losses in weight (30 to 40 per cent). The addition of small amounts of fresh cabbage or carrots or other fresh vegetables cured the animals suffering from scurvy. They drew the conclusion, which later investigators have abundantly confirmed, that scurvy was due to the absence of a certain chemical substance from the "scorbutic" diets. They were able to show that the anti-scorbutic substance is destroyed by cooking or drying.

The first to observe the development of scurvy in guinea pigs as the result of restriction to a faulty diet was Theobald Smith (1895). He described this condition as being induced by limiting guinea pigs to a diet of oats and bran. This chance observation was not followed by a detailed study and remained unnoticed for more than twenty years.

Infection Theory.—The work of Holst and his co-workers did not explain the etiology of scurvy to the satisfaction of all. Jackson and Moore (1916) made the remarkable observation that guinea pigs fed a diet of oats and milk *ad libitum* develop typical scurvy. Bolle (1902) was apparently the first to state that guinea pigs become scorbutic when fed exclusively on raw or cooked milk. Hart and Lessing (1913) produced scurvy in monkeys by feeding condensed milk, rice and dried pignuts. Milk is known to induce growth for a time in young animals during the suckling period when it constitutes the sole source of nutriment. This naturally suggested that some factor other than diet entered into the etiology of scurvy. Jackson and Moore

(1916) isolated a diplococcus from the tissues of scorbutic guinea pigs. This organism, cultivated in the laboratory and subsequently inoculated into pigs kept on a diet capable of maintaining health, produced hemorrhages. The organism was some weeks later again isolated from the lesions. These findings afforded strong evidence that scurvy may be induced by bacterial infection.

Faulty Deductions of McCollum and Pitz.—The observation that guinea pigs develop scurvy when fed on oats and milk seemed very significant to McCollum and Pitz (1917). As a result of extensive studies on the nutrition of the rat, McCollum and Davis (1915) formulated the working hypothesis that the simplest diet which can support normal nutrition in this species is one which fulfills the following conditions: It must contain sufficient protein of the "complete" type; adequate amounts of nine mineral elements in appropriate combinations (Na, K, Ca, Mg, Cl, P, S, Fe, I); a source of the carbohydrate glucose, and two dietary factors of undetermined chemical nature, which McCollum and Kennedy (1916) provisionally called fat-soluble A and water-soluble B. These were recognized as belonging to the class of food substances which Funk (1913) had designated by the term "vitamine."

McCollum had found that a diet of oats and milk sufficed to maintain a rat in a state of health for a long period. When McCollum and Pitz, independently of Jackson and Moore, observed that guinea pigs confined to a diet of oats and milk quickly succumb to scurvy, they found it difficult to believe that the disease could be due to the lack of a specific substance, for milk alone suffices as the sole food for all young mammals during a critical period of their lives. It was difficult to accept the view that anything essential for normal nutrition was absent from milk and oat mixtures.

They observed that their guinea pigs which were suffering from scurvy developed impaction of the cecum when the condition was brought about by feeding milk and oats, both of which are constipating foods. They, therefore, offered the tentative explanation that injury to the cecum of this species is of primary importance as a factor in the etiology of scurvy. They accepted the view of Jackson and Moore that the cause of the disease was an invasion of the tissues by bacteria, made possible by the damage to thececum as a result of the retention of feces. The

observation that paraffin oil or cathartics sometimes saved scorbutic animals from death lent support to this view. In the light of later studies McCollum and Pitz accepted the view that scurvy is a deficiency disease.

Early View of Hess on Cause of Scurvy.—After the appearance of the paper of McCollum and Pitz, Hess (1917) presented a study of infantile scurvy which seemed to support strongly the view that scurvy is not simply a deficiency disease. He stated that infants fed milk pasteurized at 145 degrees for thirty minutes did not develop scurvy, whereas others fed the same milk which had been allowed to age for twenty-four hours on ice did so. He stated that milk pasteurized at 165 degrees was more likely to induce scurvy than milk which had been pasteurized at the lower temperature. Hess stated that aging of raw milk increased its liability to cause scurvy in infants. The degree of heat to which the milk had been subjected seemed not to be the most important factor, for according to his statement boiled milk did not produce the disease. The most plausible deduction from these observations appeared to be that boiling tended to render the milk sterile, whereas heating to 165 degrees killed most of the lactic acid-forming bacteria, leaving the resistant spore-forming organisms in the milk. These could develop rapidly because with aging the lactic acid content would not rise and hold them in check as is the case in milk soured in the normal manner. Hess concluded that it was the staleness and the bacteriological condition, rather than the temperature to which the milk had been heated, which determined whether or not it would cause scurvy in an infant. As the result of later studies Hess (1920) abandoned this theory and accepted the view that scurvy is a deficiency disease.

The appearance of these papers aroused a number of investigators to renew the study of the etiology of scurvy, and the various theories concerning the etiology of the disease soon were subjected to the test of new experiments. Two papers, one by Chick and Hume, the other by Cohen and Mendel, deserve special consideration since they explained the errors into which several workers had fallen, and fully established the correctness of the view of Holst that scurvy is a deficiency disease.

Chick and Hume Confirm Views of Holst.—Chick and Hume (1917) extended the observations of Holst and his co-workers by making a comparative study of the values of a number of food-

stuffs as protectives against scurvy. There are two methods which may be employed for the study of such a problem. One is to employ animals of a definite and uniform size and to add to a scorbutic diet a certain amount of the food under examination to discover whether or not the animals are protected from the disease. The alternative is to produce scurvy and then attempt to relieve it by giving the food, the anti-scorbutic properties of which are under investigation. Chick and Hume adopted the preventive type of experiment. They fed a basal ration of oats and bran, which produced scurvy in about three weeks in a guinea pig weighing about 280 gm. The substance to be studied was added in known amount and the duration of the protection, if any, was observed. They emphasized the superiority of this method over the curative type. In the latter the animals return to normal slowly even when the anti-scorbutic potency of the diet is high, and their improvement is less well marked when it is low. This method leads to confusion in interpretation of results. Sherman and co-workers (1922) in their extensive experiments also used this method. They have further standardized the procedure by employing guinea pigs 6 to 8 weeks old and weighing 300 to 350 gm.

In a later paper, Chick, Hume and Skelton (1918) discussed their experimental work in relation to the views expressed by the several investigators, who held that scurvy was not a deficiency disease. In the light of their data the erroneous conclusions of McCollum and Pitz were explained. They showed that the anti-scorbutic value of milk had been overrated. They adopted hand feeding, instead of allowing the guinea pigs to eat of milk *ad libitum*, and showed that when less than 50 c.c. of raw milk daily supplemented a diet of oats and bran it did not defer death from scurvy beyond about 30 days. When 50 c.c. of raw milk were consumed daily the life of the animals was extended to about 75 days. An intake of 100 to 150 c.c. of milk daily served to maintain the guinea pigs free from scurvy for 16 weeks when the observations were discontinued. Hume (1921) states that the guinea pig requires a considerable amount of vitamin A for growth and maintenance. She believes that some of the confusion of the histological data on scorbutic diets results from shortage of vitamin A as well as C.

It is necessary, therefore, that the diet should consist almost entirely of milk if, through its agency alone, scurvy is to be prevented. Chick, Hume and Skelton point out that this con-

cept is consistent with the rôle played by mammalian milk, which is destined to form a complete food for a definite but limited period in the life of the young.

These studies offer a satisfactory explanation of the observations of McCollum and Pitz, who found that guinea pigs which were in the early stages of scurvy and at the same time badly constipated as the result of an exclusive diet of oats and milk, could in some cases be relieved by the administration of paraffin oil. Harden and Zilva (1918) pointed out that any amelioration brought about by the administration of this laxative was due to the increased consumption of milk because of the clearing of the alimentary tract, and consequent improvement in the well-being of the animals.

Studies of Cohen and Mendel on Scurvy.—Cohen and Mendel (1918) were the first to produce scurvy in guinea pigs on a diet which was complete from the nutritive standpoint except for vitamin C, and which was suitable in its physical properties for the alimentary tract of this species. Any animal which possesses so highly specialized a digestive tract as does the guinea pig, in which the stomach and cecum are very large and delicate, cannot be satisfactorily nourished during long periods on a diet which is thoroughly digested and absorbed, and which leaves little residue, or one which forms hard or pasty feces. The cereal grains leave little residue, and oats and milk form hard feces. Cohen and Mendel (1918) prepared a diet of cooked soy bean flour supplemented with 3 per cent each of sodium chlorid and calcium lactate, and dried brewer's yeast, and sufficient raw Jersey milk to furnish 5 per cent of butter fat. The yeast was added for the purpose of providing vitamin B, and butter fat to furnish vitamin A.

On this diet, complete except for vitamin C, young animals gain weight rapidly and for a time their appearance is satisfactory. On about the tenth day, while still eating well and gaining in weight, they begin to show tenderness of the wrists and ankle joints. Their condition after this time rapidly becomes worse, and the joints swell to two or three times their normal size. The appetite fails and a sharp decline in weight ensues. At this point the animals can be saved by the administration of foods containing a sufficient amount of vitamin C.

With this diet Cohen and Mendel were able to confirm the observations of Holst and of Chick and her co-workers. They describe what appears to be simple scurvy unaccompanied by

other conditions due to malnutrition. They have given the most complete description of the syndrome of scurvy in the guinea pig. Its onset is usually characterized by tenderness of the wrists, ankles and knees, developing in the order named. Within a day or two the affected joints become swollen, frequently to the extent of two or three times the diameter of the normal bone. Spontaneous fracture of the bones is of frequent occurrence. These conditions are observed especially in guinea pigs which weigh 150 to 250 gm. Older animals lose the use of their hind legs because of stiffening and paralysis. The animals find relief in lying on the side or back so as to favor the affected members. This position has been described by Delf (1918) as the "face-ache" position. If the animals are permitted to remain long on the diet deficient in vitamin C the swellings fail to disappear when the diet is corrected, and develop into exostoses. When curative measures are administered early enough the bones appear to return to normal.

Anatomical Lesions in Scurvy.—The two most noticeable conditions observable at autopsy are hemorrhage and fragility of the bones. The stomach, intestines and cecum may show congestion, hemorrhage or ulceration, but according to Cohen and Mendel these organs may in some cases be normal in appearance. These pathological conditions are usually observed in animals fed oats and water or oats and milk, and less frequently in those fed the diet of Cohen and Mendel. These investigators point out that an ever present condition in advanced scurvy is lack of appetite. They found the food intake to diminish as the disease advances, but that fasting does not produce the characteristic lesions of scurvy. These observations are in harmony with those of Lewis and Kerr (1916).

A remarkable observation made by Mendel and Cohen is that the swollen joints and tenderness often appear while the animals are still growing rapidly and have good appetites. Inanition can, therefore, play but a minor rôle, if any, in the production of the more prominent symptoms of scurvy. There seems to be regularly a period of 10 days or so of fasting previous to the death of the animals which are confined to the scorbutic diet of soy beans or cereals, for the loss in weight during this period corresponds to the rate seen in guinea pigs during starvation.

Metabolic Disturbances in Scurvy.—Several observations have been made which throw light on the nature of the metabolic disturbances in scurvy.

LaMer and Campbell (1920) found an increase in the weight of the adrenal glands which they believe may represent a compensatory response to the decreased adrenalin production known to exist in a scorbutic animal. The heart and kidneys, they believe, had increased in weight, but the liver of scorbutic animals did not appear to be enlarged.

Morel and co-workers (1921) state that the bone changes seen in experimental scurvy are not related to defects in calcium metabolism. Morikawa (1920) observed the following changes in the suprarenals in experimental scurvy in guinea pigs: Increase in weight, increase in lipoidal content of the cortex and reduction in amount of doubly refracting fat. He states that the lipoid in the zona fasiculata was distributed in 3 layers, viz., an outer layer rich in lipoids, a middle layer poor in lipoids, and an inner layer rich in lipoids. McCarrison (1921) has reported a remarkable enlargement of the suprarenal glands in the guinea pig during experimental scurvy. The changes in these organs were slight during the first fifteen days, but during this period the weight of the spleen, liver, stomach, intestines and their integument were definitely reduced. These results have been confirmed by Bessesen (1922). Wells (1921) says that the tooth pulp is abnormal in scorbutic guinea pigs. Hess, Unger and Pappenheimer (1922) found that prolonged exposure to direct sunlight did not prevent scurvy in the guinea pig. Gerstenberger and Burhams (1922) found that scorbutic infants and guinea pigs, as well as polyneuritic pigeons, can burn carbohydrates completely.

Iwaduchi (1922) found a decrease in amount of ash in muscle and bone from scorbutic guinea pigs. The fat content of muscle and adrenal was decreased. The content of calcium and of phosphorus in the adrenals was below normal. The calcium content of the blood was 50 per cent below normal. Refractometric readings showed the protein content of the blood to be subnormal. Haemoglobin was markedly decreased, although the number of red corpuscles was essentially normal. Iwaduchi, however, could not confirm the observations of others that the calcium and phosphorus content of the bone was abnormal. Smith (1923) found no evidence of deposition of calcium in the tubercle in infected guinea pigs when calcium was administered together with cod liver oil. He states that cod liver oil has a definite but slight effect on the nutrition of the non-tuberculous guinea pig.

Liotta (1923) found that there was a marked diminution in the number of red corpuscles, as well as in the amount of haemoglobin, and a slight increase in the number of leucocytes in the blood of guinea pigs in scurvy produced by a diet composed exclusively of oats. Höjer (1924) has discussed at length the pathology of the bones, teeth, muscles, heart, liver, spleen, kidney, adrenal and salivary glands, lung, blood, blood vessels and connective tissue in scurvy in the guinea pig.

Harden and Zilva (1920) found that monkeys of 2 to 3 kilos body weight were protected from scurvy by the small amount of orange juice that was required to protect a guinea pig of 300 to 400 gm., viz., 2 to 5 c.c. per day. They found that the monkey develops scurvy in about two months, whereas the guinea pig requires only about two weeks. Findlay (1921) states that guinea pigs fed a diet lacking in vitamin C do not develop scurvy more rapidly when the diet contains an excess of protein, or fat, or carbohydrate. He observed that death is slightly hastened in guinea pigs suffering from scurvy when the diet contains an excess of carbohydrate. Mouriquand and co-workers (1924) fed guinea pigs a scurvy-producing diet during 24 days. Severe acute scurvy developed. The animals were then fed an anti-scorbutic ration for from 10 to 50 days, during which they returned to an apparently normal condition. This was effected in 10 to 12 days. When the recovered animals, together with new animals for controls, were subjected to a scurvy-producing diet, the recovered animals showed symptoms of scurvy a day or two sooner than the controls. They interpreted their results to indicate that an attack of scurvy may produce changes in the histological elements of the marrow which render the animals more susceptible to subsequent attacks. Anderson and Smith (1924) conducted experiments to determine the effect of severe acute scurvy on the subsequent growth and food intake of guinea pigs. They found that the loss of body weight observed in scorbutic guinea pigs at the height of scurvy cannot be accounted for by inanition alone. Growth in the post-scorbutic stage is either parallel to or greater than that of the normal pig, and is accompanied by a definite increase of food intake.

Rat and Certain Other Species Immune to Scurvy.—It has not been found possible to produce experimental scurvy in the rat. One must conclude that the rat either does not require the anti-scorbutic substance in its metabolic processes, or has the capacity to produce the substance synthetically. If the latter

assumption be true the nutrition of this animal would be independent of this factor in the food.

Shipley, McCollum and Simmonds (1921) found the bones of rats in polyneuritis brought on by a diet adequate except for lack of vitamin B to be quite indistinguishable from those of guinea pigs dying of scurvy. The bones were very osteoporotic. One received the impression that all growth must have stopped. The epiphyseal cartilage was very shallow (only 4-5 cells in depth) and stained intensely with basic dyes. The marrow contained comparatively few nucleated cells (leucocytes and their predecessors). The blood vessels of the medullary cavity were crowded with red blood cells. In many places the vessels had ruptured and numerous small and large hemorrhages were to be found, especially in the vicinity of the cartilage. In some bones the active bone marrow had been almost completely replaced by hemorrhages. The bones of rats fed the same diet with 30 per cent of rolled oats as a source of vitamin B revealed no lesions whatever, notwithstanding the specific fasting of these rats for the vitamin C.

In order to determine which of these explanations holds for the immunity of the rat from scurvy, experiments were carried out in our laboratory in the following way: We fed young rats a diet of purified foodstuffs supplemented with a hot alcoholic extract of wheat germ to furnish vitamin B. They, therefore, did not have access to the factor C, since this is absent or nearly so from wheat germ. The rats grew to adult size, and remained in health to the age of 15 months on a diet which would induce scurvy in young guinea pigs within 10 to 25 days. At this point Parsons (1920) produced acute scurvy in guinea pigs. She then fed the fresh livers of these rats to the scorbutic guinea pigs and observed relief of the symptoms of the disease. In this manner several animals were completely cured of scurvy by the livers of rats which had had no anti-scorbutic substance in their diet. There can be but one conclusion from such a result. The rats were able to synthesize the vitamin C from their food.

Parsons and Hutton (1924) and Lepkovsky and Nelson (1924) have confirmed and extended these studies. They have carried rats into the second generation on a scurvy-producing diet, and have cured scurvy in guinea pigs by feeding them the livers of the rats. Bailey and Hyde (1923) have shown that hemagglutinins which exist normally in the blood of the adult fowl are not present at hatching, but appear at a definite time after-

wards. Those active for the rat and rabbit cells appear about the sixteenth day, whereas those active for guinea pig cells appear about 3 weeks later. It would be interesting to inquire whether the vitamin C is present in the livers of newborn rats, when it is present in the diet of the mother and when it is lacking.

Parsons and Reynolds (1924) fed guinea pigs a diet deficient in vitamin C. They found that livers taken from normal guinea pigs cured scurvy in other pigs. Livers taken from scorbutic pigs failed to cure the disease in other pigs suffering from the acute disease.

Man, guinea pig and monkey are all susceptible to scurvy because they lack the synthetic capacity possessed by the rat. Another example of a species which is able to subsist without vitamin C is the prairie dog of the western plains. Parsons and McCollum (1920) fed a young prairie dog born in captivity a diet which would produce scurvy in a guinea pig within 30 days. The animal was able to grow in a nearly normal manner and escaped the development of scurvy during a period of 10 months.

Scurvy in Rabbits.—Lopez-Lomba (1923) found that rabbits on a scorbutic diet can reproduce and induce normal growth in the young during the suckling period. They state that suckling rabbits are very sensitive to a lack of the vitamin C, and since they remain in health the milk of the nursing mother must provide this substance. They did not determine whether the presence of the vitamin C in milk could be accounted for through a large reserve of the vitamin C in the maternal organism or whether the rabbit synthesizes this substance. Lesne, Christou and Vaglianos (1923) report that they kept a pregnant guinea pig on a vitamin-free diet, giving daily 3 c.c. of orange juice subcutaneously. After 15 days young were born. The injections were thereafter given intraperitoneally. On the eighteenth day, when the secretion of milk had nearly stopped, and the young showed swelling and pain in the posterior parts, one pair was suckled by another female which was at the beginning of lactation and given a scurvy-producing diet, together with subcutaneous injections of vitamin C (orange juice). These young died of scurvy in 44 days. Another pair was suckled by their own mother and received a supplementary sterilized ration. These died of scurvy in 25 days. These experiments seem to indicate that vitamin C introduced subcutaneously or intraperitoneally is not absorbed sufficiently rapidly to make it possible to ad-

minister the vitamin through these channels. Hess and Unger (1918) reported that orange juice after it had been boiled and made alkaline to litmus by adding sodium hydrate just before using can be given intravenously to infants with marked curative effect.

Findlay (1921) fed 6 adult rabbits, 3 males and 3 females, on a diet of one part of oats and two parts of wheat bran, with an unlimited supply of water. The females became pregnant and gave birth to young. The young were almost fully developed but were born dead. On examining the 7 newly born rabbits it was found that in all the knee joints were swollen and congested, and showed signs of hemorrhage into the tissues surrounding the joints. The costochondral junctions were also enlarged in every case, while in 2 cases there was hemorrhage in the axillae. Microscopic examination of the knee joints and the tissues in relation thereto showed extensive hemorrhages into the muscles and into the synovial membranes of the joints. As in acute scurvy in the guinea pig, so here also the actual changes in the chondroblasts were not well marked, though the bone marrow was greatly congested. The internal organs also showed great congestion, while hemorrhagic foci were noted in the kidney in 4 cases, in the adrenals in 3 cases, and in the liver in 3 cases. The non-susceptibility of the rabbit to scurvy, Findlay concludes, must be looked upon as relative rather than absolute. Rabbits deprived of the vitamin C lose weight and eventually die. The addition of the vitamin C to the diet is followed by an increase in weight and an improvement in the general condition. The experiments showed that a pregnant rabbit deprived of vitamin C throughout the period of gestation produces offspring which show hemorrhages in the joints and in the internal organs, although the mother remains healthy.

Pigeons do not Require Vitamin C.—Plimmer and Rosedale (1923) kept pigeons during 15 months without a source of vitamin C. During this period the birds laid eggs and reared young. They kept chicks from one day after hatching to maturity without vitamin C. These birds laid eggs and a few young were hatched from the eggs. Ducks, geese, turkeys, guinea fowl and pheasants were reared from the day old stage to ages of 12 to 16 weeks without vitamin C. They conclude that these species of birds do not require the vitamin C. Orr and Crichton (1924) state that the requirements of swine for vitamin C are rather low and that in the interval between weaning and slaughter there

is little likelihood of pigs suffering from a deficiency under practical feeding conditions.

Chemical Properties of Vitamin C.—A number of investigators have contributed observations concerning the solubility and other properties of the vitamin C. Hess and Unger (1918) showed that when orange juice is precipitated by the addition of 95 per cent alcohol or absolute alcohol, the solution remains anti-scorbutic, but the precipitate is of no value. They also state that the vitamin is soluble in eighty per cent alcohol.

Harden and Zilva (1918) studied the effect of alkalinity on vitamin C. Orange juice made alkaline equivalent to N/20 with NaOH and stored 20 hours in a cold room did not prevent or delay the onset of scurvy when doses of 5, 6 or 7 c.c. daily were given. Samples of juice alkalinized to N/50 on the date of feeding and then fed in doses of 3, 5, or 7 c.c. did not prevent scurvy. Alkalinity is very destructive of the vitamin C. Vedder (1921) states that the anti-scorbutic principle is neither an acid nor a salt. Neutralized orange or lemon juice diluted with alcohol was effective only in a fresh condition.

Zilva and Miura (1921) studied the differential dialysis of vitamins B and C. Vitamin C from lemon juice from which citric acid had been removed by calcium carbonate failed to pass through a collodion membrane which had been soaked in 90 per cent alcohol, but did pass through a membrane soaked in 95 per cent alcohol. Both vitamins B and C diffuse through a collodion membrane of such permeability as permits the passage of substances like methylene blue, neutral red and safranin.

Hess (1921) states that copper greatly accelerates the destruction of the vitamin C, and that the small content of copper in milk which has been in contact with utensils containing this element is sufficient to catalyze the destruction of the vitamin C so that it is rapidly destroyed. Hess and Weinstock (1924) conducted a series of animal experiments on milks containing known amounts of copper. Two and one-half parts per million of copper added to milk appreciably reduced the anti-scorbutic value during heating over what took place under similar conditions without copper.

LaMer (1921) studied the temperature coefficient of the reaction in which the vitamin C is inactivated by heating, and concludes that the substance is neither a protein nor an enzyme. The rate of inactivation increases with a decrease in hydrogen ion concentration. He believes the inactivation is due to intra-

molecular oxidation and reduction such as occurs in aldehydes and sugars.

Hart, Steenbock and Lepkovsky (1922) studied the solubility of the vitamin C from desiccated orange juice. They found it soluble in 80, 95 and 100 per cent ethyl alcohol. It is also soluble in ethyl alcohol, but insoluble in butyl alcohol, benzene, petroleum ether, acetone, ether, chloroform and ethyl acetate.

LaMer, Campbell and Sherman (1922) found that reducing the hydrogen ion concentration tends to increase destruction of vitamin C on heating tomato juice.

Zilva (1922) found that decitrated lemon juice pH 6.8 could be boiled for 2 hours in an atmosphere of carbon dioxid without loss of potency, whereas in a single hour in a current of air its potency was reduced to less than 25 per cent.

Zilva (1922) observed that ozone destroys vitamin C. It can also be destroyed by passing air through a solution containing it at ordinary temperatures. Two hours' boiling in the presence of carbon dioxid did not destroy it. One hour's boiling in air did destroy it. Hydrolysis of decitrated lemon juice with hydrochloric acid for 5 hours impairs C but a considerable amount of it is still there.

Zilva (1924) concentrated decitrated lemon juice to one-tenth volume and then acidified it by adding 7 gm. of citric acid to 1 liter of juice. Fifty cubic centimeters of this concentrated juice was introduced into a series of flasks which were evacuated, sealed and stored in the laboratory in the dark. Samples were tested 3 and 5 months later and no loss of potency could be detected. He (1924) found that invert sugar in decitrated lemon juice may be fermented without appreciably altering its anti-scorbutic potency. He (1924) made decitrated lemon juice N/20 alkaline (pH-12.5) and found that when exposed to the air at room temperature it rapidly lost its anti-scorbutic potency. Decitrated lemon juice of the same reaction kept 24 hours at room temperature in the absence of air showed no deterioration. When air is aspirated through a boiling solution of decitrated lemon juice for more than 1 hour the destruction of the anti-scorbutic factor is delayed when the hydrogen ion concentration is raised from pH 6.6 to 2.2.

Zilva (1924) removed the sugar from decitrated lemon juice by fermentation, leaving an active fraction consisting of an apparently complex mixture of substances and showing an appreciable nitrogen content with a total solids of 03.-0.5 per cent.

When this fermented active juice is concentrated to a small volume and treated with absolute alcohol a precipitate is formed which is not active. Basic lead acetate also forms a precipitate in the solution thus obtained and the vitamin C is all in the precipitate. By combining these procedures a very potent preparation was obtained on removing the lead from the precipitate. Zilva found that all the active fractions reduce ammoniacal silver nitrate and decolorize potassium permanganate. The purest preparations showed an extremely low nitrogen and phosphorus content.

Tests Proposed for Vitamin C.—Bezssonov (1921 and 1922) has described the preparation of a phosphotungstic-phosphomolybdate reagent which does not give a reaction with monophenols, except a yellow brown with guaiacol, and no color with uric acid or glucose. With diphenols it gives a persistent intense blue with hydroquinone; with pyrocatechol and catechol a red violet turning to blue, but no color with orcinol and resorcinol; a yellow brown with protocatechuic acid, pyragallol and tannin. All anti-scorbutic extracts gave a blue color. After treatment which destroys the vitamin the blue color was diminished or not produced. He believes that the color is due to an unstable radical, probably one of the polyphenols which in solution is easily split off from the vitamin. Kay and Zilva (1923) studied the application of this reagent to the identification of vitamin C and found that the majority of anti-scorbutic liquids give a color reaction, but that a preparation obtained from lemon juice did not give it, although active as an anti-scorbutic. Bezssonov (1924) further discusses the application of his test. Koopman (1924) compared the *in vitro* test of Bezssonov with results of feeding experiments. The results of the comparative tests agree in general but vary with milk, the color reaction being negative but the test with the guinea pig positive. Sajous (1924) has described a test for the vitamin C. In this the food is crushed, or rasped, and its fluid is treated with a few drops of 1-1,000 adrenalin chloride solution. A few drops of a solution composed of one part of 3 per cent hydrogen peroxide solution to nine parts of water is then added. When mixed in a test tube the two solutions give no change of color. It is only when a tissue or fluid contains the oxidizable vitamin that reaction occurs. A positive test is indicated by the development of a red color. Sajous refers to this as the "adrenin H_2O_2 test."

Stability of Vitamin C.—Holst and Frölich (1912) called

attention to the fact that the anti-scorbutic potency of fruits and vegetables displays greater stability in acid than in alkaline solution. They observed that fruit juices were more heat stable than vegetable juices and suggested that the acidity protected the anti-scorbutic principle. Fürst (1912) working in Holst's laboratory found that whereas dry cereals or pulses did not prevent scurvy, they acquired anti-scorbutic properties when allowed to germinate. He was the first to point out the possibility of producing the anti-scorbutic principle at will by the germination of seeds. Wiltshire (1918) confirmed Fürst's observations on the value of germinated seeds in the treatment of scurvy.

Hess (1917) found yeast to be without value as an anti-scorbutic food for children. He used an autolyzed product and gave 15 to 30 c.c. daily. Wheat germ he found to possess some anti-scorbutic value but much less than orange juice. He points out that infants may develop scurvy while gaining steadily in weight. He found that alkalization of foods tends to destroy the anti-scorbutic value.

Delf (1918) found that when 0.5 per cent of citric acid was added to the water when germinated lentils were cooking the destruction of vitamin C was greater than when the acid was omitted.

Hess and Unger (1918) obtained good results in the treatment of scurvy in guinea pigs with canned tomatoes. They also gave 15 c.c. of canned tomatoes per day to infants of three months of age and over. They recommend this as an economic and efficient anti-scorbutic for children. Hess (1920) advises 30 gm. of canned tomatoes per day for infants. Hess and Unger (1918) found fresh orange juice to be anti-scorbutic but juice which had been preserved in a refrigerator for 3 months had lost a considerable amount of its potency. Ten minutes heating at 110° C did not entirely destroy the value of the juice. These investigators (1918) found that young fresh carrots withstood dehydration, retaining a part of their curative properties. They demonstrated the superiority of young over old carrots as a source of vitamin C. Delf (1918) compared cabbage heated at various temperatures for different lengths of time, with raw cabbage, as anti-scorbutic foods. She estimated that 70 per cent of the vitamin is destroyed by one hour's heating at 60°, and over 90 per cent in the same time at 90°. After 20 minutes' heating at 90 to 100° the loss is about 70 per cent.

Harden and Zilva (1918) made from lemon juice a prepara-

tion entirely free from citric acid which still retained much curative power for scurvy in infants, guinea pigs and monkeys.

Hess and Unger (1919) state that an infant requires fully half a pint of unheated milk daily to protect it against scurvy. Hart, Steenbock and Smith (1919) found that milk sterilized at 120° C for 10 minutes, and commercial unsweetened evaporated milk had lost their anti-scorbutic properties when used in quantities equivalent to an amount of raw milk which would prevent scurvy in guinea pigs on a diet of rolled oats and dried hay.

Zilva (1919) studied the effect of ultra-violet light on the vitamin C. Lemon juice brought to H-ion concentration of pH 2.34, and also some which was made neutral, was exposed to rays for 8 hours without influencing its properties.

Lewis (1919) found 25 gm. of banana to be the minimum protective dose for guinea pigs. Dutcher and co-workers (1919) found that raw lean beef does not possess anti-scorbutic properties sufficient to permit of its demonstration by experiments with guinea pigs.

Campbell and Chick (1919) studied the anti-scorbutic value of canned foods, sterilized by exposure to steam at 100° for one and one-half hours. This treatment reduced the value of 20 gm. of bean pods to less than that of 5 gm. of fresh material. Under comparable conditions 70 per cent of the vitamin C in cabbage was lost.

Chick and Delf (1919) again emphasized the anti-scorbutic value of peas and lentils which were soaked for 24 hours in water and germinated for 2 days at room temperature. These sprouted seeds were found to be inferior to orange or lemon juice, cabbage or swedes, but equal to green beans or potatoes, and superior to carrots or red beets in anti-scorbutic properties.

Chick, Hume and Skelton (1919) tested several dried fruits as anti-scorbutics, which have long been esteemed in India for this purpose. Tamarinds, cocum and mango possessed definite but small anti-scorbutic value.

Zilva (1919) acidified concentrated anti-scorbutic juices and stored them under anaerobic conditions. He found no perceptible loss in potency after 3 months' storage.

Harden and Robison (1919, 1920) studied the anti-scorbutic value of concentrated fruit juices. The volatile constituents of orange juice were valueless. The juice was concentrated to a syrup under reduced pressure at 40° C. One gram of this, equivalent in solids to 9 c.c. of fresh juice, when fed daily protected a

guinea pig against scurvy. The desiccated juice preserved 7 months was still potent. Concentrated lime juice was prepared by a rapid process in which heating was continued less than a minute. The loss of anti-scorbutic property was found to be slight. Apple juice concentrated to one-sixth its volume within a minute had anti-scorbutic value but the jelly seemed to deteriorate faster than orange juice.

Holst and Frölich (1920) say that thin slices of cabbage which were dried for a week in an incubator at 37°, and then at the same temperature by means of phosphorus pentoxide, showed very pronounced anti-scorbutic properties. When kept at 37° for 18 to 26 minutes in vacuum bottles the cabbage lost almost all its anti-scorbutic properties. Delf (1920) found that the juice of cabbage was much more affected by exposure to 100° C for one hour than was the juice of swedes or oranges.

Pierson and Dutcher (1920) found solid rhubarb, rhubarb juice, or the juice boiled for 15 minutes curative against scurvy in guinea pigs.

Kago (1920) investigated the anti-scorbutic value of some food-stuffs. Animals fed 15 c.c. of cabbage daily with a "bean cellulose" ration remained free from scurvy, while those fed the basal diet developed the disease in 15 to 24 days. Pickled radishes, pickled plums, bean paste, salt pork and salted salmon did not protect. Sprouting red beans were comparable to cabbage.

Bassett-Smith (1920) found lemon juice to keep in concentrated or tablet form. Boiling for 5 minutes he states did not appreciably diminish the anti-scorbutic properties. He says that tablets of dried lemon juice prepared in the cold have retained their vitamin C content over 12 months. Lemon juice loses its anti-scorbutic power unless protected from heat. It seems proven that oxidation and not heat is the principal cause of destruction of vitamin C.

LaMer, Campbell and Sherman (1920) found that boiling tomato juice of natural acidity (pH 4.2) for one hour destroyed 50 per cent of its vitamin. Four hours boiling destroyed 70 per cent.

Bezssonov (1921) found that the addition of half a gram of a mixture of citric or tartaric acids and four parts of sucrose to 200 gm. of potatoes prevented discoloring and the loss of the anti-scorbutic value during the grinding of potatoes. He also found intact raw potatoes equal to cabbage or dandelion. The marc of potato crushed and pressed in a hydraulic press had very

slight anti-scorbutic action. The juice was anti-scorbutic but less so than an equivalent amount of potato.

Givens and McClugage (1921) reported that guinea pigs are protected against scurvy by daily doses of 2½ gm. of fresh raw tomatoes, by 10 gm. heated one hour at 100°, by 2 gm. of dried tomatoes heated 15 minutes at 100°, by 10 gm. of canned tomatoes heated at 15 lbs. pressure for 30 minutes, by 3 c.c. of commercial canned tomatoes 3 years old, by 10 gm. of commercial canned tomatoes 3 years old cooked 15 minutes at 100°.

Givens and co-workers (1921) also found 10 gm. of raw apple or of bananas sufficient to protect the guinea pig against scurvy for 3 months. An equivalent amount of these foods cooked at 100° for 15 minutes or dried at 55-60°, or both dried and cooked, failed to protect the animals against scurvy. Delf (1921) says that the juice of a medium orange or lemon, or a fairly large tomato daily furnishes sufficient vitamin C for an average adult.

Givens and Macy (1921) tested the following substances dehydrated by a typical commercial process: Lemon juice raw and neutralized, grape juice, tomato juice, raspberry juice, grapefruit juice, and orange juice. All except grape and raspberry contained a significant amount of vitamin C. Dehydrated orange juice was effective after two years. All the dried juices tested except the raw lemon juice were 14 to 20 months old.

Shorten and Ray (1921) investigated carrots, brinjal, spinach, cabbage, tomatoes, potatoes, turnips, turnip tops and mixed factory dried vegetables. The materials were placed in steeping water, then transferred to a wire basket and scalded, after which they were dried in the sun. They were packed after a week's storage. In experiments on guinea pigs they found that tomatoes, potatoes and cabbage retain considerable vitamin C. Sun-dried spinach, carrots, brinjal, turnips and turnip tops had little or no anti-scorbutic properties. Mixed factory vegetables did not protect.

Davey (1921) found that oranges and lemons kept in a cool room but not frozen retained most of their anti-scorbutic property so long as the fruit was edible. Harden and Robison (1921) dried orange juice and kept it 14 months at a temperature of 29°. The material was then a hard taffy mass containing 8 per cent of water. It had lost about 85 per cent of its anti-scorbutic power. Another sample kept the same length of time at room temperature was still potent but not so good as fresh juice.

Ellis, Steenbock and Hart (1921) report that cabbage dried

in carbon dioxide at 65° for 35 hours or prepared as sauerkraut, and corn silage, were all ineffective as anti-scorbutics. Orange juice retained its vitamin C after extraction with ether, aeration for 2 hours or treatment with hydrogen. A part of the activity was removed by shaking with blood charcoal or by passing through a Chamberlain filter. It was completely destroyed by one-half volume of hydrogen peroxide or by a slight excess of potassium permanganate in 1 hour.

Anti-scorbutic Value of Powdered Milks.—Hart, Steenbock and Ellis (1921) studied the anti-scorbutic properties of milk powders. They found that aside from the factor of initial quantity of the vitamin in the milk, which varies with the season and manner of feeding of the cow, milk powders vary in their potency with the process of manufacture. The spray process is, they found, more destructive of the vitamin C than the Just process, in which the milk is dried on the drum. These experiments point out the limitations of milk powder in infant feeding. Anderson, Dutcher and co-workers (1921) report that boiled milk was found practically equivalent in anti-scorbutic properties to fresh unheated raw milk. Pasteurized milk heated at 63° C for 30 minutes did not protect guinea pigs against scurvy. The pasteurized milk had been stirred vigorously while heating, whereas the boiled milk had remained undisturbed. The pellicle formed on the surface protects it against the oxygen of the air. The destruction of the vitamin C is attributed to oxidation. Oxidizing agents such as hydrogen peroxide were shown to rapidly destroy vitamin C.

Jephcott and Bacharach (1921) compared summer milk, winter milk, and neutralized milk dried by a roller process with milk dried by the spray process. They found the milk powders prepared from both summer and winter milks by the roller process to possess anti-scorbutic values about equal to the original raw milks from which they were prepared. The neutralized milk contains less vitamin C than raw milk and the spray processed dried milk was markedly deficient in anti-scorbutic value. Cavanaugh, Dutcher and Hall (1923) say that milk powder prepared by the spray process preserves its original strength of vitamin C.

It appears that some of the experimental work on the anti-scorbutic value of milk powders has been carried out under the auspices of commercially interested people, and it is not always possible to determine what data should be given credence.

Clark and Collins (1922) conclude that milk powders and their remade products are safe for infant feeding. From the data available, however, it seems certain that infants or young children fed milk powders should receive suitable anti-scorbutic food daily.

Heat Stability of Vitamin C.—Sherman and co-workers (1922) made an elaborate study of the anti-scorbutic value of tomato juice. They found that 2 c.c. of fresh tomato juice enabled guinea pigs to live 70 to 90 days, but at autopsy hemorrhages of a mild character were seen. Growth was subnormal after 15 days and the animals showed soreness of the joints. Three cubic centimeters afforded complete protection. Seven cubic centimeters of tomato juice daily which had been heated 4 hours at 100° C was judged equal to 2½ c.c. of the raw juice.

Givens and co-workers (1922) found that raw apples and bananas possess anti-scorbutic properties but that much of their potency is lost in cooking. They found 10 gm. of raw apple or 1½ c.c. of lemon juice to be the minimum protective dose for a guinea pig. Eckman (1922) found dried peaches, apricots, apples, pears, prunes, cherries and loganberries do not prevent scurvy in guinea pigs when 4 gm. per day are fed. Peaches delay but do not prevent scurvy.

Mouriquand and Michel (1922) report that lemon juice heated for 1½ hours at 120° loses so much of its vitamin C that it requires 30 to 40 c.c. of the juice to prevent scurvy in guinea pigs. They report that 2 to 3 c.c. of fresh orange juice is sufficient. Driel (1922) describes experiments on guinea pigs which indicate that malt extract heated to 75° has no anti-scorbutic value. This would indicate that beer contains no vitamin C.

Embrey (1923) found that 10 gm. daily of pomelo, cucumber, chico and guava, protected guinea pigs from scurvy for 9 weeks, whereas 15 gm. of banana, kangkong leaves, and camote leaves were essential for similar protection. Höjer (1924) found 25 c.c. of commercial whortleberry juice failed to protect guinea pigs. Five c.c. of strawberry juice showed some protective effect. He found that 3 c.c. of the juice of tomatoes fully ripe, but produced late in the season, had very little protective effect on guinea pigs. Raspberry juice and black currant juice were of little or no value as anti-scorbutic foods.

A most interesting observation has been made by Kohman and Eddy (1924). They have shown that the destruction of the

vitamin C during the process of cooking or canning is a matter of oxidation rather than the application of heat. Apples were used in their study. This fruit contains about 5 per cent by volume of oxygen and as in other fruits a process of respiration goes on. When the supply of atmospheric oxygen is cut off the oxygen in the apple is used up in approximately 13 hours. The apples are prepared for canning by peeling and quartering, and are then covered with a weak solution of salt in water. This prevents access of air. The oxygen in the fruit is rapidly consumed and the fruit can then be canned or cooked in any way that seems desirable without loss of vitamin C. Apples prepared in this way and stored for nine months showed no appreciable loss of vitamin C. As apples when held in cold storage gradually lose their vitamin C content, canning by their method actually preserves the vitamin much better than when the fruit is kept in its natural condition. Canned spinach nine months after canning, whether processed for 70 or 120 minutes at 240° F was shown to be one of the richest sources of vitamin C yet found.

Eddy and Kohman (1924) report that feeding tests with cabbage processed by a commercial method of canning showed a loss of vitamin C which is markedly less than with cabbage cooked in open kettles. The loss appears to be due to oxidation; temperature and time of heating were not found to be factors.

Relation of Food of Cow to Vitamin C of Milk.—The studies relating to the anti-scorbutic value of milk are of special interest because of their importance in the nutrition of infants. McCollum and Simmonds (1916 and 1918) showed that neither vitamin A nor B is present in the milk unless it is supplied by the diet of the lactating rat. If this should be true of the vitamin C as well, it is obvious that the nature of the food of cows whose milk is to be used for infant feeding, when it serves as the sole source of this substance, is of great importance.

Barnes and Hume (1919), Dutcher, Pierson and Biester (1920) and Hart, Steenbock and Ellis (1920) have studied this problem. The last named have reported studies of the anti-scorbutic value of milk of cows which had been kept on dry feed for varying periods, as contrasted with that of cows which were feeding upon green pasture. Summer milk was found to be much richer in vitamin C than winter milk. Fifteen cubic centimeters of summer milk afforded protection against scurvy to a guinea pig during twenty weeks, when superimposed upon a scorbutic

diet. For most individuals it was found that as much as 50 c.c. daily was necessary to afford complete protection. Of winter milk from cows which had been fed exclusively on dry feed for many months, 70 c.c. were necessary. Silage or sugar mangels added to a ration of dry feeds did not improve the anti-scorbutic value. Dutcher and his co-workers (1920-1921) found 20 c.c. of summer milk superior to 60 c.c. of winter milk in vitamin C. When cows were changed from a ration of fresh green feed, to one of dry grains, hay and fodder, the milk did not fall off rapidly in its anti-scorbutic value, but only after the lapse of some weeks did it become inadequate. When cows were changed to a green pasture after a long period on a dry feed, their milk very promptly improved in its value as an anti-scorbutic agent. Hess, Unger and Supplee (1920) confirmed the observation that milk of cows on dry feeds is deficient in vitamin C.

Diet of Nursing Mother Should Contain Fresh Foods.—Cow's milk, and probably human milk, has relatively low anti-scorbutic value even when derived from a satisfactory diet. It suffices to protect the young when the diet is almost wholly derived from milk. When top milk is used, modified by dilution and the addition of sugar or cereal water, the infant may be in danger of scurvy. Dr. P. G. Shipley has called our attention to the fact that there are only a few cases of infants having recognizable scurvy before the age of about 9 to 10 months. The reason for this is not clear. It is not known whether the infant is born with a reserve of the vitamin C, as it is with iron, or whether it possesses in very early life the power to synthesize this substance.

Further evidence relating to the importance of the mother's diet to the anti-scorbutic value of her milk is afforded by a report made by a committee of the American Pediatric Society in 1898. Among 356 cases of scurvy in infants 10 had been nursed. The following table gives information regarding the anti-scorbutic potency of milks treated in different ways.

TABLE XV

Relation Between the Kind of Food and the Incidence of Scurvy

Breast milk	12 cases; alone in 10
Raw cow's milk	5 cases; alone in 4
Pasteurized milk	20 cases; alone in 16
Condensed milk	60 cases; alone in 32
Sterilized milk	107 cases; alone in 68
Proprietary infant food	214 cases

These data show that a small amount of vitamin C remains in heated milk. The occurrence of a small number of cases among nursed infants shows that the meat, bread and potato type of diet which has been so extensively used in this and other countries in recent times, does not suffice for the production by a nursing mother of a milk which is complete for the nutrition of her infant. We have no way of determining how large a number of infants, as a result of taking breast milk of poor quality, have reached the stage of borderline cases of scurvy, but whose conditions have not reached the point where they were clinically recognizable. There is reason to suspect that it is large.

Infantile Scurvy a Recent Condition.—Infantile scurvy is not often mentioned in early medical writings. Not until the middle of the nineteenth century did it attract the attention of medical observers. Until the last few decades infants were breast fed or else they died. Not until the eighteenth century was artificial feeding or mixed feeding mentioned in medical literature. Bread and water, called "water pap"; bread and pulse; small beer with egg, etc., were the common foods administered to an infant which had lost its mother. Usually the unfortunate one died from intestinal infection or inanition before it had time to develop scurvy.

Underwood, in 1793, was the first to use cow's milk as a food for infants in his practice as a midwife. About the middle of the nineteenth century the manufacture and sale of proprietary foods became important. Doubtless there was a parallel increase in infantile scurvy. The development of the science of bacteriology led to the discovery that typhoid fever, bovine tuberculosis, scarlet fever, septic sore throat and other diseases might be milk-borne, and health officers began a campaign for the pasteurization of the milk supplies of cities. While this practice protected adults against epidemics which had been shown to follow milk routes, it was disastrous to many infants. Pasteurized milk was deficient in vitamin C and scurvy among infants increased as the artificial feeding came more into vogue. Only through the researches of the last decade has this danger to infants become fully understood, and the means of correcting thiss error discovered.

Barlow (1894) first described a diseased condition which we now know to be a complication of rickets with scurvy in infants. The syndrome became known by his name. He states that the

disease was new in England, but more common in Germany, and that it appeared to be on the increase both in England and America. His paper is the earliest and best description of scurvy in infants, and is one of the classics of medical literature.

It is interesting to correlate the history of human scurvy with the changed conditions under which large groups of persons have lived at different times. In early times the disease affected chiefly soldiers and prisoners, since they were subjected to simple and monotonous diets consisting of dry, cooked or stale foods. These foods keep well, and are selected by those who purvey for large masses of people, especially when transportation or storage is an important matter. With the advent of steam navigation no special precautions are necessary for the protection of sea-faring men against this disease, since they frequently have access to fresh food.

Investigations as to the cause of scurvy gives us another example of animal experimentation contributing a blessing upon the human race. Without systematic experiments with the guinea pig years or decades would certainly have passed before the cause of scurvy would have been understood. Thenceforward we may confidently look for the protection of babies, soldiers, explorers and prisoners, as well as the general population, against this malady.

CHAPTER XII

THE DIETARY DEFICIENCY DISEASES (*Continued*)

BERI-BERI AND POLYNEURITIS

Antiquity of Beri-Beri.—Beri-beri is a disease of great antiquity, which was for a long time confined to Japan, the Dutch East Indies, Malay Peninsula, Philippine Islands, India and Southern China. Cuerva (1923) says that the first information about the disease dates from 2600 B. C., when it was already known to the Chinese. The next mention of it is in the second century A. D., when a Japanese described beri-beri as kak-ke, a name still in use in Japan. It was believed at one time to be a tropical disease, but this view has been disproved by its frequent occurrence in recent times in other places, especially in the subarctic regions, notably Newfoundland, Labrador and Norway. It is generally believed to be due to faulty nutrition and will occur in any part of the world if man restricts his diet to certain articles of food. In 1914 there was an outbreak of beri-beri in the pail at Elizabeth, N. J., caused by the poor diet of the inmates.

Symptoms of Beri-Beri.—Beri-beri is a form of peripheral neuritis, the nerves of motion and sensation being chiefly affected. It occurs in two forms, the wet and the dry. Its incipiency is manifested by fatigue and depression, numbness and stiffness of the legs, and more or less edema of the ankles and face. In the dry type, wasting, anesthesia and paralysis are the chief manifestations. The most marked symptom in the wet type is edema, which is sometimes excessive, affecting the trunk, limbs and extremities. In both forms there is generally tenderness of the calf muscles and a tingling or burning sensation in the feet, legs and arms. Fernando (1924) says that laryngeal symptoms in the form of hoarseness and often aphonia have been observed in both infants and adults. In infants aphonia has been observed so frequently that it is one of the most characteristic symptoms of infantile beri-beri. It is observed especially in the latter stages of the disease. In adults it is not so frequent, although it

occasionally occurs. Vedder (1913) says of aphonia in beri-beri "The speaking voice is lost and the patient can only whisper." Bontios (1642, quoted from Fernando) said: "While suffering from this disease the sound of my voice was so feeble for a whole month that those sitting next to me could not understand me." Vedder believes that aphonia in this disease is "probably due to degeneration of the pneumogastric nerves, thus causing paralysis of the laryngeal muscles." The mortality in this disease is usually high.

Theories as to Etiology of Beri-Beri.—Various theories have been advanced to explain the etiology of beri-beri. Since polished rice is poor in protein, fat and phosphorus, the prevalence of beri-beri among rice eating peoples has been attributed to the low protein content of the diet, to fat starvation, to starvation for organic forms of phosphorus, or to intoxication caused by a poison assumed to be present in rice under unfavorable conditions. Climate, arsenical poisoning and specific infection have been proposed as etiological factors for this disease. Many now believe that the disease is the sequel to specific starvation for the vitamin B. The history of the study of this disease by various investigators forms a chapter of absorbing interest in preventive medicine.

Study of Human Beri-Beri by Takaki.—The most notable among the early investigations relating to the etiology of beri-beri is the work of Takaki (1887), Director-General of the Japanese Navy. About the year 1880 the disease became a scourge among Japanese sailors. Between 1878 and 1883 the average number of cases treated medically in the navy was 323.5 per 1000 per year.

The principal components of the diet of the sailors were polished rice and fish, the remainder being derived from a number of vegetables. Takaki was impressed with the differences in the diet and in the health of the British sailors as compared with those of Japan, and suspected that beri-beri might be due to lack of protein. He commissioned a training ship to make a nine months' cruise, giving the common sailors a diet containing 91 gm. of protein per day. The better fed officers did not suffer from the disease, whereas the men were afflicted. There were 169 cases of beri-beri among 276 men during this cruise.

Another ship was then commissioned to make a similar voyage with the protein raised to 155 gm. per man per day. Among the crew of 276 men there were but 14 cases of beri-beri. This

experience led to an improvement of the diet of the navy, and to the disappearance of beri-beri from the service. More meat and vegetables were added to the diet and rice was replaced in part by wheat ro barley. A pint and a quarter of milk a day was furnished each man. This was made possible through the employment of condensed milk. The condensation of milk on a commercial scale began in England in 1856. Not only did beri-beri become rare, but diseases of the digestive tract and of the respiratory system became less prevalent and diseases of the eyes were diminished to half the former incidence. From the eye conditions reported it seems probable that the men were suffering from lack of vitamin A as well as vitamin B. The Japanese officials were of the opinion that the increased meat consumption was responsible for the decrease in beri-beri.

For many years this experience of the Japanese navy was regarded as a demonstration of the importance of a protein-rich diet. That this was not the cause of the immunity of the sailors to beri-beri is clear from the history of the disease among the crews of Norwegian vessels. In the invigorating climate of the northern seas, beri-beri suddenly became common among the Norwegian sailors after 1894, whereas before it had been unknown. Before 1894 their diet consisted of bread made from rye flour, milled without the removal of the germ, peas and salted meat, mostly fat pork. After that year it consisted of white bread made from bolted wheat flour, peas, salt pork once or twice a week, preserved meat and fish three or four times a week, and dry fish once a week. The addition of meat and fish in liberal amounts, although increasing the protein consumption, was nevertheless followed by the sudden appearance of beri-beri, a new disease in that part of the world. Holst (1911) tells of one sea captain who refused to eat white bread, and provided for his own table a supply of the customary rye flour bread. When beri-beri attacked his crew he was able for a time to relieve his men by sharing his brown bread with them.

Classic Experiments of Eijkman.—In 1889 Eijkman, Director of the hygienic laboratory at *Batavia* (Dutch East Indies), observed a disease among the fowls kept at his laboratory, the symptoms of which were very much like beri-beri and the pathological anatomy showed the same degeneration of nerves. By several series of experiments he succeeded in showing that this polyneuritis gallinarium occurred when the feed of the fowls consisted exclusively of completely polished rice, so-called white

rice, in which the pericarp and the germ or embryo (called together silverskin) were wanting. The disease could be cured by adding silverskin to the white rice.

Eijkman's first interpretation of the experiments was that the silverskin of the rice kernel contained some substance which neutralized the injurious influence of the starch-rich diet. To quote: "Aus den diesbezüglichen Versuchen zog ich den Schluss dass in den Silberhäutchen wahrscheinlich ein Stoff oder Stoffe vorhanden sind, wodurch der schädigende Influss der stärkehaltigen Nahrung neutralisirt wird."

Eijkman also studied the influence of climate as a factor in the etiology of the disease, but found it to be a negligible factor. In the large cities of the East Indies (Batavia, Soeralaja), 66.7 per cent of the people suffered from beri-beri, as against 30.9 per cent among mountain dwellers. The greater number of the first group ate milled rice. Prisoners fed principally on milled rice showed an incidence of 80 per cent near the sea, as compared with 62 per cent in the mountains. In one prison 5.8 per cent of the inmates suffered from beri-beri when supplied with polished rice, but the disease disappeared when undermilled rice was substituted.

Eijkman also proved that feeding exclusively with other starch-rich foods, such as tapioca, ambon sago and sago of the arenga-palm (Arenga saccharifera) also produced polyneuritis gallinarum. Potato meal was an exception, since with this food paralysis did not occur. He further showed that meat could be given as a curative treatment and to a certain extent as a prophylactic against polyneuritis gallinarum. Eijkman concluded that the cause of polyneuritis gallinarum lay in the diet, in which certain food-stuffs contained in silverskin (pericarp and germ) were wanting. He came to the conclusion that the protective substance was a normal constituent of the animal organism.

Vorderman, * Sanitary Inspector of Java, in a conversation with Eijkman, expressed the belief that in his experience in several native prisons in Java where white polished rice (rice without silverskin) was provided, beri-beri occurred among the inmates, but he believed the disease had not occurred in those places where they were supplied with red rice (rice with silverskin).

Eijkman recognized the importance of this communication, since it made possible without difficulty, experiments to show

* Personal communication from Dr. Hulshoff-Pol.

whether rice with silverskin had the property of preventing beri-beri. He induced the government to direct Vorderman to examine into the influence of rice-feeding in connection with the prevention of beri-beri. The report of Vorderman appeared in 1897, and it was proved that among 96,530 native prisoners, three-quarters of whose diet consisted of unpolished rice, only 9 cases of beri-beri occurred (0.009%). On the other hand, among 150,226 native prisoners who took white rice, or rice without silverskin, there were 420 cases (2.79%).

Although this report clearly showed the advantages of the silverskin, it provoked criticism since observations only and no conclusive experiments had been made. Hulshoff-Pol, in commenting on the situation says: "As often happens in such cases. criticism went much too far, people overlooking the good results of the work and emphasizing the weak points of it only." The physicians in the Dutch Indies at that time were convinced that the possibility of an infection was not to be excluded, so Vorderman's views did not find acceptance and his ideas were nowhere applied, except in a few prisons. Eijkman was nominated professor in the Utrecht University (Holland), 1898, so his researches were continued by Grijns.

Laurent (1899) attributed beri-beri to lack of sufficient fat in the diet. He thought the beneficial effects of the addition of meat to the diet of the Japanese sailors was due to the increase in the fat content in the food. Montgomery Smith (1899) supported the infection hypothesis. He cited the ship *Lodestar* as having a bad reputation for beri-beri during successive years while the southwest monsoon blew.

After several series of experiments Grijns came to the conclusion that other food-stuffs too had an influence on the origin and the cure of polyneuritis gallinarum. Grijns proved that a special kind of beans "katjang hidjoe" (phaseolus radiatus) had a strongly prophylactic and curative effect on this disease of fowls. Grijns further worked out the idea of Eijkman that the silverskin contained a substance which prevents and cures beri-beri. He called it a "deficiency" disease, since it was due to a deficiency of unknown substances. Grijns (1901), in interpreting his experiments, concluded that in several articles of natural food there exist substances which cannot be dispensed with without remarkable injury to the peripheral nervous system. He stated that the "katjang-hidjoe" bean is rich, and polished rice poor in this substance.

Hulshoff-Pol says: "The official inquiry of Vorderman, not having the confidence of the physicians, who were quite wrong of course, most of them still being of the opinion that no prophylactic and curative remedy against beri-beri existed, the influence of these beans on beri-beri was examined by them." In 1901 Hulshoff-Pol made experiments at the lunatic asylum at Buitenzorg (Java). He added 150 gm. of boiled "katjang-hidjoe" to the white rice, which he gave during 9 months to 78 lunatic natives; to 222 controlled lunatic natives he gave only white rice. Not one of the 78 persons who ate the beans had beri-beri, while no less than 68 of the 222 who did not eat them developed beri-beri. This experiment was repeated, but with a change of persons experimented upon, and again it was proved that the beans had a prophylactic effect. Their curative power became evident when the patients who had beri-beri because of an exclusive diet of white rice, were completely cured after 150 gm. of "katjang-hidjoe" had been given daily to each.

Manson's Studies on Beri-beri.—Manson's studies (1901) convinced him that the disease was due to a toxin elaborated by a germ growing outside the body. He suggested that improper handling of rice led to its infection and the development of a toxin. He opposed the theory that meat was a protection against beri-beri and cited the experience of the Singapore jail, where in 1898-99, there was an ordinary and a penal diet. The former contained wheat flour and fresh meat. The penal diet contained no meat. The greater number of cases of beri-beri occurred among those inmates of the prison having meat in their diet. McLeod (cited by Manson) mentions a case where beri-beri attacked the officers on shipboard, but not the common sailors. The former had such luxuries as haddies, clams, oysters, lobster and salmon. It was evident that lack of protein could not be the cause of the outbreak.

Manson also cited the observation of Hirota, who saw 52 instances of the disease in nursing infants whose mothers were suffering from beri-beri. When these infants were weaned and fed condensed or fresh milk they recovered promptly, whereas five not so treated died. It was argued by Manson that a child acquiring a germ disease from the mother will not recover so rapidly, but that one intoxicated by taking poisonous milk would be expected to do so. This he interpreted as supporting his theory of intoxication.

Mention should be made of the view of Sir Ronald Ross (1901)

who believed that beri-beri was due to arsenical poisoning. Symptoms of peripheral neuritis have been observed in persons who drank beer containing arsenic.

Hulshoff-Pol's researches were not accepted by the physicians in the Dutch Indies until 1907 when he succeeded in curing beri-beri by giving patients a decoction of the beans. Since he had extracted a crystalline material from the decoction the Government ordered an official inquiry as to the value of the beans as a remedy for beri-beri. This research, executed by Kievit de Jonge, completely proved that the beans were curative. There have been no experiments on humans with the crystalline material (X-acid), since the quantity obtained was too small, but it has been shown with fowls suffering from polyneuritis gallinarum that the crystals produced a cure. Thus in 1907 in the Dutch Indies the curative value of the silverskin was not yet generally accepted, although it had been fully demonstrated experimentally that a remedy against beri-beri had been found in katjang-hidjoe.

Studies on Human Beri-beri.—Fletcher (1907 and 1908), at the Kuala Lumpur Lunatic Asylum in the Federated Malay States, carried out a thorough experiment on human subjects to determine the relation of rice to beri-beri. He divided the hospital into two sections, each containing nearly equal numbers of patients. To one group he supplied milled rice, and to the other "cured" rice. "Cured" rice is prepared by soaking unhusked kernels, or paddy, in water, then heating the soaked grains until they burst. The rice is then dried in the sun. This rice is husked and ground for use without polishing. The kernel, including the germ, was supplied to half the patients. The diet of all the inmates consisted of fresh meat, fresh fish, salt fish, curry stuffs, coconut oil, rice and vegetables daily. The only difference in the diets of the two groups of patients was in the character of the rice.

The diets were continued for a year with the following results. Many in the group eating the milled rice developed beri-beri, whereas no cases developed in the group fed the steamed rice. Fletcher mingled some of the beri-beri patients with the well and continued the experiment. The result was a demonstration that the disease was due to the consumption of a diet consisting in great measure of meat, fish and milled rice. We now know that the same result will follow the too free use of endosperm of any of the cereal grains as food.

Toxicity Theory.—One of the hypotheses as to the cause

of beri-beri was that food-stuffs contain something toxic. Schaumann (1908) was unable to detect any poison which might have been produced as a metabolic product by a microbe. He found the urine of patients did not contain a toxin. He suggested that beri-beri was due to starvation for certain organic phosphorus compounds.

In 1910 Fraser and Stanton repeated the experiments of Hulshoff-Pol and proved that "cured rice" or rice with silverskin was a prophylactic for beri-beri. Their experiments had, however, been preceded by those of Fletcher (1906-7).

Since the early experiments of Schaumann there had been a tendency to associate beri-beri with Barlow's disease. Kohlbrügge (1911) connected these diseases with unbalanced diets too rich in starch. He believed such diets favored the growth of fermentative bacteria which formed acids and tended to self-sterilization of the intestine, depressing or extinguishing the normal flora. He designated them "fermentation diseases." He believed organisms occurred in rice and other cereals, and regarded them as related to acetic acid formers. He postulated that a diet containing free acids tended to hold the injurious bacteria in abeyance.

Funk (1914) observed that the symptoms of beri-beri did not appear in fasting birds and that an increase in the amount of food ingested, especially of carbohydrate, hastened the onset of the disease.

Schaumann (1914-15) demonstrated that dried codfish and pickled meats, heated at 120° C, produced polyneuritis. Dogs, cats, rats, a goat and an ape were used in his experiments. He produced the disease in these animals, but not in mice. The latter suffered loss of weight and showed signs of malnutrition.

In the early experiments the special importance of the germ of seeds was not recognized. The cortical layer was regarded as the one of importance. McCollum and Davis (1915) in their study of the wheat kernel first called attention to the fact that the germ is exceedingly rich in the substance curative for polyneuritis. They suggested that it is the loss of the germ rather than of the cortical layer which robs rice of its protective power in beri-beri.

The view that beri-beri is due to deficiency of phosphorus in the diet was supported by Edie and Simpson (1911). Polished rice and refined cereal foods contain little phosphorus, and this suggested the phosphorus starvation theory. In the light of

later experimental studies, these seemingly irreconcilable observations have been made clear.

Schaumann's Activator.—Schaumann (1915) showed that pressed yeast, wheat bran and dried testicle were all capable when added to such a diet of protecting animals against polyneuritis. Other observers added egg yolk, beef heart, etc., to the list of foods protective against beri-beri. Schaumann supplemented milled rice with purified protein and mineral salts, but these did not prevent the disease. These observations, and the failure of inorganic phosphates to protect against beri-beri, confirmed his belief that there was a protective substance in certain foods to which he gave the name "activator." He correlated the high content of organic phosphorus in rice polish with its curative power. He believed this disease was a specific starvation for this "activator," which he thought to be an organic compound of phosphorus. This view was recognized by the Far Eastern Association of Tropical Medicine. It adopted a resolution that rice containing less than 0.4 per cent of phosphorus should be excluded from those countries in which rice formed the principal food. The view that a lack of phosphorus compounds caused beri-beri was, however, soon to be abandoned.

Carbohydrate Consumption and Beri-beri.—Vedder (1918) found that the rapidity with which polyneuritis develops in pigeons bears no relation to the amount of rice eaten but depends upon idiosyncrasy of the birds. He stated that experiments performed to determine the relation of the anti-neuritic vitamin to carbohydrate metabolism by hand-feeding animals with varying amounts of carbohydrate are fallacious, for the amounts fed the animals are large or too small. The birds fed too much die from overeating and those fed too little of starvation. He states that fowls fed sterilized meats or sterilized eggs develop polyneuritis. When fed equal parts of sterilized meat and rice or of sterilized eggs and rice they develop polyneuritis more slowly than when fed on sterilized egg or sterilized meat alone. Vedder believes that the anti-neuritic vitamin is not concerned with carbohydrate metabolism.

It is easy now to understand how so many different views as to the cause of beri-beri could arise. The observations were in themselves generally reliable but it was not possible to interpret them because nobody understood what factors were necessary for an adequate diet, and the specific rôle they played. The existence of the vitamins, while hinted at by the prevailing views

on scurvy, and by early students of beri-beri, were on the whole practically unappreciated. The recorded speculation regarding them was nebulous. The demonstration of the existence of such factors by crucial experiments on animals was a matter of great difficulty as has already been pointed out in the second chapter. The history of beri-beri serves as an example of the inability of pathologists or epidemiologists to solve the problem of the etiology of such a disease. It illustrates the value of fundamental chemical research directed toward the solution of the problem of the chemical complexes which can serve as the simplest adequate diet for the normal nutrition of man or animal.

McCarrison (1924) insists that beri-beri is not strictly a disease due to food deficiency. He says that in country districts about Madras, India, the home pounding of rice is still the common practice, and beri-beri occurs among the users of home pounded or unpolished rice in endemic areas. The disease, he says, is about ten times less common among the users of this rice than amongst the users of polished rice in these areas. But while it is not true of endemic areas of the Madras Presidency that beri-beri never appears when undermilled rice only is used, it is true of non-endemic areas. He further states that undermilled and parboiled rices may and apparently do afford protection against beri-beri outside the endemic zone but they do not always afford protection in the endemic area. This points to the fact that possibly some factor other than diet may be of importance in these areas.

McCarrison says that contrary to the usually accepted belief, Mohammedans in endemic areas of the Madras Presidency suffer more from beri-beri than Hindus, notwithstanding that the food of the former is much more varied, and that the latter restrict themselves much more rigidly to a rice dietary. When he made this observation it seemed to him increditable, and it was not until he found that Malcolmson, Geddes, Macdonnell, Calhoun, and others had also made it nearly a century ago that he was satisfied as to its truth. In regard to the matter of caste, Pariahs, who eat anything, suffer most from beri-beri in endemic areas of the Madras Presidency; native Christians, who have no food scruples, suffer next in order of incidence of the disease; Mohammedans, who eatmost things, come next; and Hindus, the greatest rice-eaters of all, last in order of affliction by beri-beri. He also states that out of a total of 565 cases of beri-beri occurring among Indian troops located in the various military stations throughout

India during the years 1900 to 1914, or 89 per cent, were in Madrassis, and the majority of these in men recruited from the northern coast division of Madras where beri-beri is endemic. He states that facts such as these almost force us to the conclusion that there are factors concerned in the causation of beri-beri in endemic areas—apart from the too exclusive use of a rice dietary—which are absent or of feeble potency in non-endemic areas. Experimental evidence also supports the view that residence in endemic areas imparts to certain individuals a susceptibility to this malady which they take with them to non-endemic areas. McCarrison's observations in this regard support those of Malcolmson in 1835. McCarrison says that there is no evidence in Madras that the rice exported from that area has anything to do with the spread of beri-beri. Parboiled rice had the pericarp attached to the grain and so preserved much of its vitamin B. He has fed rice from different places to pigeons and has had varying results. He obtained pigeons from different places and also had varying results with the number of pigeons which developed beri-beri. It appears from his work that the pigeons coming from regions where beri-beri was endemic were more susceptible to beri-beri than pigeons from areas where beri-beri was not common.

McCarrison suggests that it is possible that certain of his pigeons were carriers of an invisible virus which exercised a specific effect on the epithelial tissue. The fact that beri-beri developed only in birds obtained from a certain place is presumptive evidence in favor of such a view. The gastro-intestinal flora of the men suffering from beri-beri may have been of such a nature as caused the evolution of a poison which exercised a specific effect on the heart. It may be that certain birds were of such a constitution that the disturbed metabolism resulting from the vitamin insufficiency caused a poison to be evolved in those which suffered from beri-beri which was not evolved, or not to the same extent, in those which escaped. Whether the poison which imparts to polyneuritis columbarum the characteristics of true beri-beri be an extrinsic or an intrinsic one, McCarrison is of the opinion that such a poison exists; that it is one peculiar to certain places or is evolved in persons residing or who have resided for considerable periods in certain places; and that its operation is rendered possible by the insufficient intake of a certain vitamin or vitamins in an otherwise ill-balanced diet excessively rich in starch and deficient in suitable proteins. The

conclusion which he reached in 1919, that vitamin deficiency is rarely the sole agent responsible for the causation of beri-beri, has been strengthened by his later investigations.

Beri-Beri and Vitamin B Starvation.—Clair (1920) believes the condition produced in pigeons by feeding polished rice to have no connection with beri-beri. He believes the disease in man is due to toxic products of bacterial decomposition of rice. He regards it as an infectious disease of unknown nature. Oseki (1921) asserts that the essential difference between human and avian beri-beri is that the former is due to an insufficiency and the latter to a deprivation.

Hofmeister (1922) says that a comparison of the pathological symptoms and anatomical findings on rats fed deficient diets indicates that in rat beri-beri there is no evidence of degeneration of peripheral nerves. Hence this cannot be designated as true polyneuritis. In all severe cases there were alterations in the central nervous system such as hemorrhage in the cerebellum and medulla and consequently degeneration of the neural elements. The spread and number of hemorrhages were unmistakably related to the intensity of the nervous symptoms observed. The pathological, anatomical diagnosis, he says, is not polyneuritis but cerebral purpura similar to hemorrhagic encephalitis which appears in chronic poisoning with alcohol, lead and arsenic preparations.

Shinza (1923) reported the production of a condition in rabbits closely resembling experimental beri-beri when the animals were given excessive amounts of sodium salts. This he attributes to the withdrawal of potassium from the body. The addition of potassium bicarbonate to the food resulted in a decided lengthening of the period necessary for the appearance of the symptoms of avitaminosis, whereas sodium bicarbonate had no such effect When animals were suffering from beri-beri they could be cured, he states, by the administration of a potassium salt, whereas the provision of a sodium salt made their condition become steadily worse.

Ogato and co-workers (1923) state that the syndrome developing from a deprivation of vitamin B and that of beri-beri seem different. They state that vitamin contents of the viscera of beri-beri cases are not different from those of normal viscera. In polished rice disease the nervous symptoms develop toward the end of the course of the disease, while in beri-beri they develop earlier. The blood pictures do not agree. In vitamin B depriva-

tion there is anemia with a numerical decrease, atrophy and plasma-cell degeneration of the lymphocytes. Therefore the pathogenesis of beri-beri lies, they believe, not in deprivation of vitamin B in the tissues but in the toxin production by intestinal bacteria. Okada and co-workers (1923) state that in 7 out of 10 cases of vitamin B starvation the basal metabolism was decreased and resembled the normal value on administration of vitamin B. In beri-beri the basal metabolism was usually normal except in a few cases where there was marked diminution. The diminution in basal metabolism was accompanied by paralysis or atrophy. Beri-beri is not, they believe, due to vitamin B starvation.

Wet Beri-Beri, War Edema and Atrophy.—It has been mentioned that beri-beri occurs in two forms, the wet and the dry. Those suffering from the dry type are usually at least somewhat emaciated, whereas those suffering from wet beri-beri are dropsical. Until very recently there was no clue to the cause of this difference. A brief history of the observations leading to its explanation will be of interest.

Hirsch (1885), in his handbook of Geographical and Historical Pathology, mentions edema in the legs, in the serous cavities, and in the lungs in some cases of beri-beri. Several other investigators mention edema with cases of scurvy. Gibson and Conception consider hypertrophy of the heart and edema typical of infantile beri-beri, and mention edema in beri-beri in dogs and pigs. McCarrison (1919) reports edema in pigeons fed polished rice and also after starvation.

There are recorded many epidemics of dropsy occurring in times of food shortage owing to famine conditions brought on by drought, flood or war. These have been called epidemic dropsy, war dropsy, and wet beri-beri. In the famine of 1876-77 in India, nine-tenths of all deaths were due to famine dropsy, dysentery, diarrhea and debility. The government attempted to operate a system of rationing by determining the calorific needs calculated on the basis of body weight. This system proved a failure because those who appealed for relief were found to be either emaciated or apparently very fat. The latter appeared to be in no immediate danger of starvation, yet they were in many cases in imminent danger of death. They were not fat but dropsical. The contrast between one group of the population which was nothing but skin and bones, and another which was bloated, occurred in persons of all ages.

Wagner (1887) reported an epidemic of edema among infants in Germany caused by feeding modified milk alone or with cereal. Patterson (1899) described cases of edema among starving Chinese. Weed seeds and greens obtained from wild plants were their only food. In 1917 the City of Mexico was under military rule for several months and many cases of dropsy developed. Beets and spinach were almost their sole food supply. Potter (1904 and 1912) described an epidemic of dropsy among infants restricted to barley water, which was very low in protein, inorganic and fat content. Czerny and Keller (1906) speak of "Mehlnährschaden" which is literally a state of malnutrition resulting from too much cereal in the diet. Such a diet is too rich in carbohydrate, too low in protein and in fat. There are, we now know, serious mineral deficiencies, lack of vitamins A, B and C in all cereal preparations, especially when these represent essentially the endosperm of the seed.

Holst (1911) states that many cases of dropsy occurred during the Crimean war when there was an epidemic of scurvy. He states that dropsy occurs every year on French fishing vessels off the coast of Newfoundland. Edema was very common among prisoners in England and in America during the first half of the nineteenth century. This happened before prisoners received the humane treatment and moderately satisfactory diet now provided. It was recognized, as some of the names indicate, that it had its origin in faulty nutrition. Budzynski and Chelchowski (1916) described "hunger swelling" during the war in Poland.

Edema has been frequently associated with beri-beri and scurvy. The cases recorded point to a low protein dietary as the condition favoring the development of the disease. Experimental data seem to prove that protein starvation is the specific cause of the condition, but a water-rich food especially promotes the development of edema. Denton and Kohman (1918) restricted young rats to a diet of carrots and found that a large percentage of them suffered from edema. Kohman (1920) extended these experiments by feeding carrots supplemented with purified food substances, and found that edema could be produced only in the animals confined to an inadequate protein intake and given somewhat excessive amounts of water with their food. Maver (1920) refers to the occurrence of edema in horses and oxen which work about sugar factories and subsist

FIG. 8.—Shows edema in a baby fed too largely on skim milk and cereal foods. This condition is frequently associated with incipient ophthalmia of dietary origin.—Courtesy of Dr. Bloch.

for a time upon sugar cane. Cattle fed distiller's wash, or too liberally on beet pulp, become dropsical.

Pathology of Nutritional Edema.—Weltmann (1921) says in regard to the pathology of famine edema: "Three cases of the disease showed symptoms indicating pancreatic insufficiency, as diarrhea, steatorrhea and kreatorrhea. The duodenal juice showed almost no tryptic activity and also a marked reduction in lypolytic and amylolytic activity. Autopsy showed marked atrophy of the pancreas. Alimentary deficiency in persons of weak constitutions increases the hypopancreatism and a vicious cycle results."

It seems plausible that in wet beri-beri, protein starvation is superimposed upon a deficiency of vitamin B. Wet beri-beri may be a double deficiency, whereas dry beri-beri is believed by some to be due to a single deficiency. This is doubtless rarely the case, since a diet which produces beri-beri is so poor in quality with respect to several factors that it cannot be regarded as a syndrome of an uncomplicated nature. It is this fact that explains the tendency of students of the diseases of dietary origin to emphasize the points of similarity of the two "deficiency" diseases, scurvy and beri-beri. Dilatation of the right side of the heart is reported for both scurvy and beri-beri (Hess, 1917). Vedder (1916) points out striking similarity in the nervous symptoms observed in scurvy, beri-beri and pellagra, and notes that there are certain resemblances in their symptomatology and pathology.

Kobayashi (1923) says, unilke other edematous diseases, *e.g.*, nephritis, nephrosis, the edema fluid contained as little as 0.2 or even 0.1 per cent of albumin. The consistency of the blood was nearly normal. This indicates that beri-beri is a disturbance of the tissue and not of the circulatory system.

Ashford (1922) reports cases of beri-beri in the garrison at San Juan, Porto Rico. The pathological condition was a form of beri-beri complicated by glandular deficiency. This, he believes, was induced by protein deficiency in the food. The ration furnished sufficient vitamin B, but contained too much rice and fat and too little fresh meat and vegetables. The natural food habits of the native soldiers led them to eat enormous quantities of the polished rice. Mann (1920 and 1924) described a condition among prisoners in Haiti which has many features like beri-beri, and which appears to be due to faulty diet. There is a wet and

a dry form of the disease. Mann mentions the following features of the disease which seem to differentiate it from true beri-beri.

Beri-beri	Edema Disease of Haiti
Cardiac hypertrophy.	No case of cardiac hypertrophy seen in nearly 200 autopsies. The heart is usually reduced in size.
Palpitation and rapid pulse rate.	Palpitation not marked; slow pulse rate, usually around 50 per minute, as low as 28 per minute recorded.
Motor paralysis sometimes present.	Only one case of paralysis was discovered.
Vomiting sometimes present.	Vomiting never detected.
Fever sometimes present.	Fever usually absent. Temperature subnormal.
Loss of appetite.	Appetite increased and perverted.
Symptoms required longer period to develop.	Symptoms develop more rapidly, often within 3 weeks after exposure to the etiological factor, and as early as 10 days.
Steppage gate.	Not present.

Vedder (1923) states that there is a strong possibility that beri-beri may be caused by the lack of two vitamins, and that dry beri-beri is caused by the deficiency of the anti-neuritic vitamin, while wet beri-beri, epidemic dropsy and prison dropsy are caused by the deficiency of a second as yet unnamed vitamin.

Nature of Vitamin Action.—Walshe (1918) states that there are two factors in the production of beri-beri, the absence of a vitamin and the use of certain foods which are the direct and immediate cause of the disease. He suggests that in the absence of a specific vitamin, carbohydrates undergo an aberrant hydrolysis with the production of toxic by- and end-products, thus producing beri-beri. The disease would appear to be an intoxication. A consideration of the physical and chemical properties of vitamins suggests their relationship to enzymes, and their being concerned with the hydrolysis of carbohydrates. He calls attention to the fact that starved fowls live sufficiently long to develop beri-beri, yet they die without developing it although they have been without the necessary vitamin. Seidell (1921) apparently accepted the view of Walshe (1918) that vitamins play the part of enzymes. Seidell compared them as

regards occurrence, stability to heat and alkali, solubility relations, dializability, loss of activity in fractionating proceedures and amounts necessary for causing transformations, and concluded that there is no reason why they should not be classed with the enzymes.

Green (1918) says the daily requirement of pigeons for vitamin B varies with the exogenous metabolism. There appears to be no reason to think that vitamin consumption is related to carbohydrate metabolism any more than it is to protein or fat. Funk (1919) tried the effect of various substances known to influence carbohydrate metabolism on the body sugar, liver glycogen, onset of beri-beri, loss of weight and length of life in normal pigeons and in others fed polished rice exclusively. The substances tried were dextrose, phloridzin, adrenalin, thyroid, parathyroid and pituitrin. He believed that the anti-beri-beri vitamin played an important rôle in carbohydrate metabolism. Vedder (1918) has given experimental evidence which disposes of this view (see p. 207).

Catalase Content of the Tissues in Polyneuritis.—Dutcher (1918) was of the opinion that the carbohydrate effect was due to an overloading of the oxidative mechanism of the body rather than to a specific relation between the metabolism of carbohydrate and vitamin, the latter being used up in the process. He also found (1920) that in specific fasting for vitamin B the accumulation of incompletely metabolized products may affect the nervous system and account for the loss of function. He further stated (1920) that the anti-neuritic substance functions as a metabolic stimulant, since the body temperature fell during the development of avian polyneuritis and rose after giving vitamin B. He observed the catalase content of the tissues of birds suffering from the disease to be decreased to 56 per cent of the normal. It returns to normal when they are given vitamin B. He interpreted these results as indicating a reduction of the oxidation processes in polyneuritis. Such a depression of oxidation results, he believed, in the accumulation of toxic metabolic products, which affected the nervous system.

Haussler (1920) gave a review of the theories of the action and of the chemical nature of the vitamins. Wright (1921) believed that vitamin B acts by facilitating the efficient carrying out of the functions of the intestinal canal. The main effects produced by absence of vitamins, viz., diminished food consumption, loss of weight and ultimate death, he thought to be due to

intestinal stasis and absorption of toxic products which result therefrom.

Burgi (1921) stated that the influence of large doses of morphine on the respiration of rabbits can be suppressed by the administration of vitamin B without influencing the narcotic effect. The advantage of certain drugs or extracts of drugs over the pure substances isolated therefrom may, he suggests, be brought into relation to the presence of vitamins in such extracts. He suggested (1921) that vitamins act as specific stimulants to the parasympathetic nerves and to the respiratory centers. He believed that they affect the heart unfavorably. Son Blohm, Santesson and v. Euler (1921) regard the vitamins as substances related to the group of coenzymes or enzyme activators. They state that vitamin B possesses anti-neuritic action; biocatalyzer preparations either accelerate the growth of yeast or its fermentation activity. Bickel (1922) asserts that vitamins do not influence either the digestion or absorption of food material, but in the absence of vitamins the body cells gradually lose their ability to resynthesize and store the food digestion products.

Tscherkes (1922) reported experiments on pigeons which indicate that the intermediary metabolism of carbohydrates and protein in the absence of vitamin B takes on an aberrant character in that not only the tissues are incapable of utilizing the food but the food is also a poison for the organism. He fed (1923) 5 pigeons with 50 per cent of the required calories: (a and b) as sunflower seed oil; (c and d) as heated barley; (e) as unheated barley. C and d died first without exhibiting polyneuritic symptoms, while b later showed symptoms. He concludes that on a dynamically inadequate carbohydrate and protein diet death from avitaminosis is accelerated.

Respiratory Quotient in Beri-Beri.—Mattill (1923), from the data on the respiratory quotient of rats on rations with and without vitamin B, concludes that this substance is not related to the metabolism of carbohydrates, since after feeding cane sugar or glucose the quotients rose in both cases, slightly less rapidly in the vitamin-deprived group, due probably to delayed absorption. Wollman and Vagliano (1923) say the parenteral introduction of vitamin B obtained from yeast into rats provided with vitamin-free diets containing cod liver oil as a source of vitamin A is much less effective than when given by mouth. Hofmeister (1923) regards vitamin B as an electronegative compound, since it can only be absorbed in acid solutions. It is

freed from the absorbent only by extraction with an alkali solvent. Gross (1924) states that absence of vitamin B will produce intestinal stasis in the rat. Autolyzed yeast has no effect on the isolated rat intestine, whereas absence of vitamin A caused a hurrying through of the intestinal contents.

Vitamin B in Relation to Appetite.—With a view to discovering the peculiar rôle of the vitamin B in metabolism, Karr (1920) studied the effect of specific fasting for this substance upon the desire of dogs to take food. He fed diets composed of isolated food substances and essentially free from vitamins A, B and C. Commercial casein and wheat gluten were employed as sources of protein. The dogs were restricted to these diets until they refused to take the food. Then a source of vitamin B was fed separately. Yeast, milk, tomato and a concentrated extract of vitamin B were employed for this purpose. The addition of these substances resulted in prompt response of the appetite. Karr concluded that there was some relation between the desire for food and the amount of vitamin B ingested. Brewery yeast was found much more effective than baker's yeast for this purpose. He also studied (1920) the metabolism of dogs deprived of vitamin B by making quantitative studies of the assimilation of nitrogen. No decrease in capacity for digestion, nor in the character of the metabolic products eliminated could be detected as the result of specific starvation for this substance. He found that vitamin A had no such effect in influencing the appetite as did vitamin B. Cowgill (1921) confirms Karr's view that "There is some relationship in dogs between the desire to partake of food and the amount of so-called water-soluble vitamin ingested." He found that alcoholic extracts of wheat embryo, rice polishings and navy bean, all of which contain vitamin B, improved the appetite.

The views of Kennedy and Dutcher (1922) are of interest in this connection. They believe that the effect of vitamins is not necessarily one of appetite stimulation but rather a stimulation of metabolic processes which promote growth or normal functioning. This appears to be a much more logical view.

Are Vitamin B and Secretin Identical.—Voegtlin and Myers (1919) called attention to the fact that vitamin B reacted to chemical reagents such as methyl alcohol, silver, lead and barium salts in a similar way to secretin. Secretin preparations from the duodenum of hogs relieved to some extent the neuritic symptoms, and the vitamin B from brewer's yeast on injection

into dogs stimulated the pancreatic and biliary secretions. A yeast preparation which had lost its curative powers for avian polyneuritis was devoid of any stimulating effect on the pancreatic secretion and bile flow. Anrep and Drummond (1921) found that a yeast extract does not cause secretion of pancreatic juice as does secretin. Secretin can be extracted from the intestine of a cat showing polyneuritis. The suggestion of Voegtlin and Myers that vitamin B and secretin are identical is not supported. Downs and Eddy (1921) reported that rabbits made anaemic by a diet of polished rice recover more rapidly when a daily subcutaneous injection of secretin was given together with an adequate mixed diet than did the controls on the mixed diet alone. The number of red cells was not increased by injection of vitamin B preparations. Secretin and vitamin B therefore appear not to be identical.

Cowgill (1921) reports that intravenous injection of extracts of rice polishings, of wheat embryo, of navy beans, of yeast and of neutralized tomato juice which contained vitamin B did not stimulate the flow of saliva as does pilocarpine. Extracts so administered induced recovery of the appetite. Cowgill and Mendel (1921) stated that extracts of yeast, rice polishings, wheat embryo, navy beans and tomato, although containing vitamin B, were without noticeable effect on the rate of flow of pancreatic juice, bile or saliva in the dog. The intestinal mucosa of polyneuritic dogs was shown to contain secretion. They found no direct relation between vitamin B and the secretory function of the pancreas, liver and salivary glands. The hypothesis that vitamin B stimulates these glands is not supported by the experimental results. Cowgill (1922) gave dogs a diet lacking in B. Vitamin B given by a stomach tube caused a prompt recovery of appetite, which lasted from 4 to 19 days. Commercial extract of beef had no such effect. A neutral, aqueous solution, protein free, of vitamin extract, was given intravenously, and promptly restored the appetite and was followed by a complete cessation of the nervous symptoms. He says (1923) that absence of vitamin B from the diet might inhibit or check the so-called hunger contractions and so influence appetite. He has unpublished data which show that "While the motility of the stomach is affected after a time by vitamin B deficiency, this relationship is not definite enough to warrant the conclusion that disappearance of hunger contractions is the immediate cause of appetite failure, the lack of vitamin B serving as the remote cause."

Histamin and Vitamin B.—Boyenval (1922) stated that on injecting histamin into rats fed polished rice he found no effects on the cachectic progress of the disorder. On the other hand the histamin-treated rats did not show the usual premortal nervous disturbances observed in the control animals. He thought histamin may exercise an anti-neuritic effect. Koskowski (1922) confirmed the findings of Boyenval. Although histamin stimulates the activity of the digestive glands, especially of the gastric glands, and thus aids in the digestion of rice, it does not supply the lack of nitrogenous substances found in the pericarp of the rice grains and it cannot, therefore, replace the anti-neuritic vitamin.

Catalase and Vitamin B Deficiency.—Dutcher (1920) stated that the anti-neuritic substance functions as a metabolic stimulant, since the body temperature fell during the development of avian polyneuritis and rose after giving vitamin B. He reported that the catalase content of the tissues of birds suffering from the disease was decreased to 55 per cent of the normal. It returned to normal when they were given vitamin B. He interpreted this as indicating a reduction of the oxidative processes in polyneuritis. Such a depression of oxidation, he believed, resulted in the accumulation of toxic metabolic products which affected the nervous system and brought about the typical paralysis and other symptoms. Studies made by Burge (1916-17) of the catalase content of the tissues of several species of animals (goose, pigeon, sparrow) indicate that it may vary in individuals of the same species and of similar weights and in a normal nutritive condition by as much as 100 per cent. It seems questionable whether one is justified in attributing much significance to change in the catalase content of the tissues of animals as an index to their capacity for oxidation.

Stehle (1919) considers the variation in the catalase content of the blood and tissues to be satisfactorily explained as being due to variation in the number of red cells present. He regards the glyoxalase content as of no significance as an index to biological oxidation. Findlay (1921) reported a study of the effect of deprivation of pigeons of the vitamin B on the content of glyoxalase in their tissues. Glyoxalase is an enzyme which plays a rôle in the metabolism of carbohydrate. It has the power to transform "glyoxals" into lactic acid. The "glyoxal" which serves in the animal body as the intermediate substance between glucose and lactic acid is known as pyruvic aldehyde. Glyoxi-

lase is more abundant in the liver than in other tissues. Findlay found the amount of glyoxalase in the livers was reduced about one-half in polyneuritis. On inducing a "cure" by the administration of the vitamin B the glyoxalase rose rapidly to two-thirds of the normal amount.

Damianovich (1921) reported that a diet deficient in vitamin B leads to a reduction of the amount of catalase in the liver. Burge and Leichsenring (1922) found the ingestion of a large meal of meat produces an increase in the catalase parallel with the increase produced in metabolism. Burge (1922-23) concluded that a high protein diet produced an increase while a low protein diet and starvation produced a decrease in the catalase content of the entire animal. He says this is in keeping with the fact that a high protein diet increases metabolism while low protein and starvation decrease it.

Pathology of Polyneuritis.—A new conception of the pathology of polyneuritis and other "deficiency" diseases was introduced by McCarrison (1919). The most striking manifestations of polyneuritis are the loss of the coördinating powers of the muscles. The onset of the disease is generally preceded by the bird sitting with ruffled feathers, and with the appearance of illness. There is progressive weakness, and when disturbed there is a tendency for many pigeons to be taken with convulsive seizures in which they turn "cart-wheels" backwards at intervals until they die. In the acute type of the disease many birds sit with the head greatly retracted. These symptoms generally led investigators to the acceptance of the view that the lesions in beri-beri were principally situated in the nervous system. His studies reveal the fact that injury to the nerves is much less pronounced than injury to certain other tissues.

McCarrison observed functional and degenerative changes in every tissue of the body. The thymus, testicles, spleen, ovary, pancreas, heart, liver, kidneys, stomach, thyroid and brain underwent atrophy, the severity in his cases being in the order named. The adrenals suffered hypertrophy. This was associated with a proportionate increase in the content of the glands in adrenalin. Edema was invariably associated with hypertrophy of the adrenal glands, suggesting its relation to an excessive production of adrenalin. Inanition gave rise to adrenal hypertrophy and to atrophy of other organs, the brain excepted, similar to that observed in birds fed solely upon polished rice. Rice is deficient in all known vitamins, in protein and in several inor-

ganic elements. The gastric, intestinal, biliary and pancreatic disorders of birds fed only polished rice were more serious than the nerve lesions. This diet gave rise to congestive and atrophic changes in all the coats of the intestine, especially the duodenum; to lesions in its neuro-muscular mechanism; to impairment of its digestive and assimilative functions, and to failure of its protective resources against bacterial infection. Guinea pigs restricted to a diet of oats and autoclaved milk developed lesions of the digestive tract analogous to those seen in pigeons fed polished rice. Owing to the multiple deficiencies of the rice diet, it is not possible to decide as to the specific effects of deficiency or lack of a single dietary component. It is interesting to note, however, that on a diet deficient in vitamin C, guinea pigs developed lesions comparable with those found in birds with diets faulty in several respects.

McCarrison (1919) fed pigeons a diet of polished rice, butter fat and raw onions. This diet was deficient in the anti-neuritic substance and in mineral elements, especially calcium, phosphorus, sodium, chlorin and potassium. The butter fat supplied the factor A; onions the factor C, the anti-ophthalmic and anti-scorbutic substances respectively. Typical symptoms of polyneuritis appeared, showing that onion does not contain much vitamin B. Atrophy of the bowel in these birds was usually less marked than in birds on the rice diet alone. Congestion was less prominent, and the atrophy of the myenteron and of the elements of the mucosa were often comparatively slight. Two of twelve birds examined had bacterial infection of the bowel.

In a number of McCarrison's polyneuritic birds the blood was found to be infected by B. suipestifer, B. pyocyaneus, or another organism not identified. He pointed out that systemic infection was rendered easier by the presence of the pathological processes existing in the intestine as the result of dietary deficiencies. Such invasion was favored by the impaired production of digestive juices, by the malnutrition of the secretory cells owing to the continued congestion of the mucous membrane, by the increased leucocytic traffic in microorganisms between the mucous membrane of the intestine and the blood, by the greater opportunity which the debilitated mucous membrane provided for the growth of microorganisms on its surface and within its substance, and to actual breaches of continuity in the walls of the bowel itself. The imperfect digestion in the upper part of the tract offered a favorable medium for the growth of bacteria.

Unwholesome products tended to further debilitate the mucosa, and to increase the prospects of invasion by microorganisms. Infection of the blood from the bowel under such conditions would be expected and was repeatedly demonstrated by aerobic culture of the heart blood.

In guinea pigs restricted for a time to a diet of oats and autoclaved milk, McCarrison also observed localized destructive changes in the mucous and underlying coats of the stomach and duodenum. These changes were manifest before the animals had developed any of the characteristic naked eye appearance of scurvy, and he regarded them as pre-scorbutic.

Injury of Nervous System Less Than of Other Tissues.— McCarrison further pointed out that the nervous symptoms of polyneuritic birds may be ameliorated or recovered from and yet the birds may die as the result of the gastro-intestinal lesions. He emphasized that the rapid recovery from the nervous symptoms indicated that of all the tissues, the nerves are least affected as regards organic lesions by vitamin deficiency. More serious damage resulted to the intestine, and recovery was correspondingly slow. The remarkably rapid disappearance of convulsive seizures, of astasia and of cerebellar symptoms, indicated clearly that specific fasting for the anti-neuritic substance was in some manner intimately concerned with the functioning of the nervous system. This may be an activating or energizing action on the nerve cells, or a necessary condition for the completion of the nervous current. In the absence of this substance the nerve cells are incapable of normal activity, and when it is provided normal functioning is reestablished with dramatic suddenness. McCarrison accepts the view, expressed by Bayliss, that the function of the anti-neuritic substance is that of a catalyst.

McCarrison (1922) suggests that subminimal provision of vitamins results in marked impairment of the neuro-muscular control of the intestine. Consequent upon this there is failure of the latter to transport its contents in a normal manner, due to impairment of the assimilative, secretory and protective functions. He suggested lack of sufficient vitamins as an etiological factor in certain pathological states in human subjects which appear to be the counterparts of these experimentally induced states in animals.

Pigeons fed polished rice frequently suffer from diarrhea, and this symptom of vitamin deficiency disappears on administering alcoholic extracts of egg yolk. Diarrhea and colitis are almost

constant precursors or concomitants of "war edema." Subminimal dietetic deficiency of certain sorts may lead to a state of chronic gastro-intestinal catarrh in which mucous in abnormal amounts is produced by the intestine. Mucous disease is very common among European children in India who are fed largely upon sterilized milk, artificial foods, white bread, polished rice, butter, overcooked vegetables and excessive quantities of sugar. Therapeutic experience has shown that this malady yields to a rationally selected dietary. The mucous stools, the diarrhea, the marked irritability, the unhealthy appetite rapidly disappear on such a regimen, as do also the "night terrors" from which such children frequently suffer (McCarrison).

Celiac Disease.—In the category of syndromes caused in children by feeding insufficient diets, McCarrison also ventures to suggest "celiac disease" described by Still (1918). It is thus characterized: "Absent in breast-fed children, its onset between the age of nine months and two years, the diarrhea which so frequently precedes it, the cessation of growth, the ill-formed, pale, 'oatmeal stools,' the frequent association of scorbutic symptoms, the abdominal distention, the afebrile nature of the malady, the diminished size of the liver, the blood changes, the occurrence of edema, the thin bones, the muscular feebleness—all these find their counterpart in pigeons fed on an exclusive dietary of autoclaved milled rice."

McCarrison also draws a parallel between the conditions observed in animals on faulty diets and the anatomical factors seen in chronic intestinal stasis. Keith (1918) regards defective action of the abdominal musculature, and a lesion of the neuro-muscular system of the intestine, as the two factors of primary importance in the causation of intestinal stasis.

Defects in McCarrison's Diets.—McCarrison is hardly justified in speaking so unqualifiedly of "vitamin deficiency" as the cause of the lesions observed. The extensive studies on rickets have clearly shown the serious consequences of deprivation of certain mineral elements, notably calcium and phosphorus. McCarrison's diets were almost without exception deficient in several inorganic elements, including calcium, phosphorus, sodium and chlorin, and contained a decided excess of acid over basic radicals. Deprivation of chlorin quickly leads to inability of the stomach to secrete hydrochloric acid, and this alone would seriously interfere with digestion, and with the suppression of bacterial growth in the alimentary tract of which the normal

intestine is capable in some degree. Diets so deficient in mineral salts, as polished rice; rice and butter; rice, butter and onion, might well lead to a state of depletion of the alkali reserve in the blood. Such deficient diets may account for the air hunger which he attributed to acidosis brought about by acid fermentation resulting from a diet too largely derived from carbohydrate. The conditions which he described, however, are induced by dietaries of a type which is at present common in Europe and in America. Aside from details which may require revision, the conclusions of McCarrison represent an important advance in our understanding of the relation between faulty nutrition and physical deterioration.

Does Beri-Beri Occur Uncomplicated with Scurvy.—In the light of the description which has been given of the dietary properties of natural food-stuffs and the products made from them, it is instructive to study the deficiencies of typical diets which have been described as having caused many cases of the deficiency disease. In the Burma prison there was an epidemic of scurvy with the following dietary (1914, Bull. of Tropical Diseases).

	Oz. Daily		*Oz. Daily*
Rice (husked)	24	Condiments	0.125
Beans	4	Fish paste	0.5
Vegetables (kinds not specified)	10	Salt	0.25
Oil (vegetable)	0.5		

Fleming (1910) reported epidemics of scurvy in mines in Southern Rhodesia where the men were given the following foods:

Mealie meal (milled maize)	2 lb. daily
Meat	1 lb. weekly
Beans	2 lb. weekly
Monkey nuts	1.5-2 lb. weekly
Salt	ad lib.

Chamberlain (1915) reported that among 5,000 Philippine Scouts there were always from 100 to 600 incapacitated with beri-beri. Their diet had consisted of the following foods:

	Oz. Daily
Beef	12
White flour	8
Potatoes or onions	8
Polished rice	20

On changing the diet by substituting 16 ounces of unpolished rice and 1.6 ounces of beans for the 20 ounces of polished rice, and including 20 ounces of sweet potatoes, the number of cases of beri-beri had decreased by the end of 1910 to 50. The following year there were 3 cases; in 1912 but 2, and no cases in 1913.

Hehir (1919) reports that the British troops during the siege of Kut-el-Amara from December to April, 1916, used white flour or biscuits, tinned meats and horse flesh as food. Many cases of beri-beri were reported. Gastro-intestinal symptoms and marked distension of the abdomen were present in many cases. No scurvy developed. The Indian troops used dal and either atta or barley flour, but refused to eat fresh meat. They were free from beri-beri but suffered severely from scurvy. The British troops were protected from scurvy by the vitamin C of the fresh meat; the Indian troops from beri-beri by the nature of their cereal diet.

Voegtlin (1920) selected the following diet (Diet A) for 100 patients in the Pellagra Hospital at Spartanburg, S. C., who were suffering from mild and uncomplicated pellagra. He regards this as typical of the food supply of numerous homes where cases of pellagra are observed.

DIET A

Wheat bread	300 gm.	Pork	50 gm.
Butter	30 "	Potatoes	150 "
Cabbage	100 "	Prunes	30 "
Corn meal	50 "	Turnip tops	100 "
Ham	25 "	Sugar	40 "
Hominy	75 "	Milk	40 "
Corn syrup	30 "		

Patients restricted to Diet A failed to improve or grew noticeably worse. They were later changed to Diet B, a similar diet but one which contained more milk, meat and eggs. Most cases showed definite improvement in 2 weeks, and in 2 months or more were pronounced cured, except in a few more severe and advanced cases. The therapeutic value of the dietetic treatment was very spectacular.

It is impossible to say, from a study of these diets, why the Burma prison diet and the Rhodesia miners' diet should produce scurvy rather than pellagra. This is especially true of the latter diet. It seems equally remarkable that the diet reported by Chamberlain caused beri-beri rather than pellagra, for so far

as one can judge from feeding experiments with animals there are no special qualities in beef which could differentiate it from other foods in respect to any dietary essential which we should have reason to believe would induce a specific syndrome. The recent work of Goldberger suggests that a shortage of one or more of the amino-acids may determine the results. The possibility is not excluded that pellagra cannot be produced in those species of animals which have been studied because of the presence of synthetic powers not possessed by man. The proof that the rat and prairie dog on certain diets can synthesize vitamin C is an illustration in point.

The specimen diets given above have another meaning. On any of these diets a man would suffer from several deficiencies, and where scurvy was observed it seems evident that there must also have been a serious shortage of the vitamin B and consequently a tendency for symptoms of beri-beri to develop. Furthermore, the diets listed are all decidedly deficient in one or more inorganic elements, notably calcium. In these diets poor in meat there was also a shortage of phosphorus as measured by the requirements of the rat.

It seems probable, therefore, that in many instances where one of the diseases, beri-beri or scurvy, is diagnosed, the other exists in a latent stage. This would account for the points of similarity in the manifestations of the two diseases as they have been described by several observers. It also raises the question as to whether the specific effects of lack of the vitamin C or of the vitamin B, the remaining components of the diet being of optimal quality, can be said to be fully known in the human subject.

The addition to a diet producing beri-beri in the Orient of some article of food which will prevent beri-beri, will in many instances not correct the inorganic faults. From the standpoint of public health, therefore, it is by no means sufficient to supply the vitamin B (*e.g.* tiki-tiki extract). This may be done and yet the population may be poorly nourished. The infant mortality may be high because of the poor quality of the mother's milk, and adult members of the population may still early develop signs of senility. These statements are made on the basis of the assumption that the same physiological limitations with respect to nutrition which apply to animals apply also to man, and that these run parallel when the comparison of an animal with a man on the same diet is made on experiments covering

equivalent fractions of the average span of life of which each is capable.

Normal Versus Optimal Nutrition.—The tendency of those who have investigated the relation of the diet to disease has been to assume that a diet which does not lead to clinically recognizable symptoms of malnutrition is an adequate one. Now that we understand so much about the nutritive needs of the body and about the properties of food-stuffs we appreciate the existence of many grades of malnutrition. It is becoming constantly more evident that even a slight departure from the optimal in the composition of the food may lead to states of nutritional instability which become contributing factors to physical breakdown when hygienic factors are unfavorable, or when infectious processes are operating. Even under the most favorable living conditions, however, there can be little doubt that under such circumstances the body tissues lose their vitality and the span of life tends to shorten.

Nucleopast.—McCarrison regards the term "polyneuritis" as applied to the morbid state caused by feeding pigeons polished rice as a misnomer, since it suggests that the nervous tissues are exclusively or mainly involved in the pathological process, whereas no organ or tissue escapes injury under such a diet. The glandular organs and the organs of digestion are more seriously damaged than is the nervous system. He states that the morbid condition resulting from (among other things) lack of what has been called the anti-neuritic vitamin, is not in reality a neuritis, and the term "anti-neuritic" is inaccurate. He regards the term "vitamin" inapt. He conceives the nutrient principles in the class which now generally passes under this term as "certainly nuclear ingredients essential for the nutriment of the living nucleus. They are 'nuclear nourishers' without which multiplication of cells does not occur. The term *nucleopast* (that which feeds the nucleus) might well be applied to them."

Exercise and Vitamin-Deficient Animals.—McCarrison says that human beri-beri is brought on more acutely by physical exertion. Yoshiue (1922) studied the effect of exercise on rats on a diet containing vitamins and on a vitamin-free diet. Duration of life was observed and serial sections were made of the cord. The results showed that avitaminosis brought about severe functional disturbances in the central and peripheral nervous system as in other organs. The exercised rats, without any appearance of muscular paralysis, died sooner than the others.

He believes that avitaminosis lays the foundation for the development of damage to the organism when stress is put upon it. Keith and Mitchell (1923) placed rats in cylindrical wooden cheese boxes making 13 revolutions per minute. The rats traveled at a rate of half a mile an hour with rest periods between the periods of activity. They exercised between 30 to 40 hours a week. They report that exercise did not uniformly affect the well-being of the rats on complete rations or of those on rations lacking vitamin B. They indicate that the exercise distinctly hastened the appearance of the symptoms of vitamin A deficiency and hastened the death of young animals. They claim to present evidence that muscular exercise increases the demand for vitamin A.

Blacklock (1924) discussed the lack of exercise as a determining factor in the epidemiology of beri-beri. He stated that "beriberi is notoriously a disease which occurs in institutions and in places where a fixed diet and some degree of confinement are almost constantly present—for example, prisons, asylums, ships, etc. It is important for those in charge of institutions to understand that such useful occupations as tailoring, mat weaving and basket making are not such as to provide sufficient exercise." He believes "that lack of exercise accelerates and accentuates the ill effects of diets deficient in vitamin B."

Effect of Starvation on Development of Polyneuritis.—Eijkman and van Hoogenhuyze (1916) pointed out that starvation contributes to the development of polyneuritis in birds as well as deprivation of vitamin. They also insist that the hypothesis of a neuritic poison in the diet or a toxin originating from the food in the intestinal canal as probably causative factors of polyneuritis must be abandoned. Lumiere (1920) reported that anorexia in polyneuritic pigeons seemed due to the insufficiency of the functioning of the glands of internal secretion whose normal activity appeared due to the presence in food of substances which excite it. He believed that the vitamins intervened to fill the excito-secretory rôle as well as to maintain the tonus of the organs of digestion. Findlay (1921) stated that the main clinical symptoms of avian beri-beri are all referable to a functional paralysis of the central nervous system. He stated that the changes produced in fowls and pigeons by polished rice are similar to those of starvation, but may be differentiated by the evidence of nuclear degeneration, chromotolysis in the cells of the central nervous system and the retention of lipoids in the

adrenal cortex. As contrasted with normal birds, the liver and brains of birds with beri-beri show a decrease in their nucleic acid content more marked in the liver than in the brain.

Tissue Respiration in Polyneuritis.—Hess (1921) asserted that the condition which develops in pigeons as the result of a vitamin-free diet is due to the depletion of the respiratory enzymes of the tissues, and that the pathological picture of pigeon beri-beri can be reproduced by means of hydrocyanic acid which inhibits tissue respiration. Magne and Simonnet (1922) reported that pigeons on a diet deficient in vitamin B showed a lowering of the respiratory quotient characteristic of starved animals. Digestion was markedly affected and the defect in absorption could in part be explained by the low respiratory quotient. Parenteral administration of glucose showed that there was also defective tissue oxidation. Gulick (1922) stated that the deficient calorie intake must be chiefly responsible for the lowered metabolism noted on a beri-beri diet. Abderhalden (1922) reported that symptoms of disease following the ingestion of a vitamin-free diet are always preceded by a fall of temperature of the body. He found the metabolic rate of normal tissues only slightly increased by the addition of vitamin-containing preparations. He concluded that vitamins are in some way related to the oxidative processes of the cells. Caridroit (1922) found that when pigeons are fed polished rice the respiratory metabolism remains fairly constant for the first week and then drops greatly. The respiratory quotient is at first 0.9 in both controls and tests, falls to 0.7 and is then increased to 0.8 at the moment of seizure. There is a marked decrease in nitrogen elimination. The injection of histamin does not modify the respiratory coefficient.

Anderson and Kulp (1922) report that the first apparent effect on poultry of a diet of polished rice is a loss of appetite. Food consumption decreases until finally the food is refused entirely. Loss of weight is gradual but continuous and a similar decline in heat production occurs. The fall in the intensity of the metabolism is coincident with the decrease in food consumption but they believe it depends upon some factor outside of mere voluntary consumption of food because if the chickens are forcibly fed the utilization of such food is much delayed. The continued lack of vitamin B in the diet evidently causes a serious impairment of the digestive functions which during polyneuritis results in a practical cessation of digestion and assimilation. In vitamin

starvation there is no noticeable change in the respiratory quotient. During the first 3 or 4 hours after feeding rice, quotients approaching or exceeding unity were observed until a short time before polyneuritis developed. About 18 hours after feeding rice the average respiratory quotients ranged from 0.73 to 0.82. The metabolism sinks to a very low point when polyneuritis has progressed so far that symptoms of paralysis appear. The respiratory quotient seldom rose above 0.75 during this stage of polyneuritis, although the crop contained much undigested rice, indicating an almost complete inability at that time to utilize this food. Undigested rice was found in the crop and gizzard more than a week after the last feeding. The heat production falls to 50 per cent or more below the normal basal metabolism. After the animal recovers from polyneuritis the metabolism and the heat production rise rapidly but the appetite remains poor and the gain in weight is very slow.

Fleming (1923) made a metabolism study of patients suffering from beri-beri. The basic metabolic rate of these patients was the same as in normal controls. The respiratory quotients were likewise normal while the proportion of heat obtained from the oxidation of the various food-stuffs indicated a normal utilization of the food ingested. There was no evidence that a high carbohydrate diet was a cause of the disturbance. Blood sugar values for those patients were normal while determinations of blood uric acid, urea and non-protein nitrogen showed normal values in beri-beri cases. He interpreted these to mean that there was no damage to the excretory power of the kidney in beri-beri even when edema was present. Ahlgren (1923) asserts that tissue respiration of rats subjected to deficiency of vitamins A and B is not diminished, but that the tissues of pigeons subjected to vitamin deficiency show a low respiratory activity which he believes is not due to an insufficient supply of oxidizable material.

Water Content of Body in Polyneuritis.—Krause (1922) found there is no definite increase in the water content of the organs of beri-beri chickens except possibly in the skin. In beri-beri pigeons there is an increased water content in the intestine, the heart, the lungs, the skin, muscles and kidneys. Starvation in proportion to the loss in weight in birds leads to a similar increase in water in the tissues. In beri-beri rats there was no regular increase in the water content of the tissues. In scurvy in guinea pigs increased water content was not detected in separate organs. Morinaka (1922) reported the water content of the

body of mice deprived of vitamins to be the same as that of normally nourished mice. The ash content of undernourished animals was higher than that of the mice which had vitamins in their diet. The ash of the mice on the deficient diet contained a higher percentage of calcium and magnesium and a lower percentage of phosphorus and potassium.

Changes in Internal Organs in Avitaminosis.—Abderhalden (1921) found that pigeons fed 2 weeks on polished rice had marked alimentary dystrophy. Simonnet (1921) showed that pigeons dead as a result of a deficiency of vitamin B usually showed no marked changes in the weight of liver, though in some cases there was distinct relative enlargement. The pancreas showed a marked reduction, connected perhaps with decreased amylolytic activity. The adrenals showed no certain weight changes. The thyroid appeared always pale and deficient in volume. The thymus was absent. The diet employed was also deficient in vitamin C but Plimmer and Rosedale (1923) have found that vitamin C is not essential in the diet of birds.

Tiger and Simonnet (1921) stated that the pancreas of polyneuritic pigeons showed a slight reduction in diastatic activity insufficient to account for the symptoms induced by the diet, and probably secondary, developing from chronic avitaminosis. Ozawa and co-workers (1921) found that rabbits fed a vitamin-free diet showed no change in the maltose content of the liver and only a slight liver autolysis, whereas in a guinea pig on a vitamin C free diet the maltose in the liver decreases and the autolysis was high. The autolysis of the muscles increased in both animals. The amino-acids in the blood of the guinea pig increased. Findlay (1921) contrasted the livers and brains of normal birds with those in beri-beri. The latter showed a decrease in nucleic acid, most marked in the liver. The administration of vitamin B to a bird suffering from polyneuritis is followed by a removal of lipoids from the adrenal cortex, an increase in the nucleic acid in the brain, the reappearance of the Nissl granules, and the disappearance of the paralytic symptoms. He concluded that vitamin B is an essential element in the synthesis of nucleic acids by the body. Rothlin (1922) found the content of diastase in the pancreas of beri-beri pigeons to be the same as that of normal pigeons.

Abderhalden (1922) observed that pigeons fed polished rice and treated with either the tissues of normally fed pigeons or with the tissues of polyneuritic birds react in the same manner.

The alimentary dystrophy is, he says, the same in birds fed rice as in those fed purified food-stuffs without vitamins. Artom (1923) found that the cells of the pancreas of polyneuritic pigeons appeared full of zymogen granules and that the ducts were filled with stagnating secretion. During starvation the zymogen was reduced and the ducts contained no secretion. Korenchevsky (1923) states that the most characteristic changes in the glands of internal secretion in pigeons suffering from beri-beri are: hypertrophy of the suprarenals, atrophy of the thymus and spleen, with persistence of the islets of the germ centers often accompanied by an increase in their number and size and frequent hypertrophy and hyperplasia of the islet cells. Adachi (1923) followed the uric acid and allantoin excretion in 3 dogs until death occurred on the forty-sixth, fiftieth and one hundred and third day of avitaminosis respectively. In the last animal the uric acid increased markedly as avitaminosis developed; the increase in allantoin excretion was not so great. In the other two experiments the results were not so clear. He concludes that "The increase in uric acid excretion depends on a disturbance in the metabolism of endogenous purines."

Rohr (1923) found that tissues may be arranged in the order of decreasing intensity of respiratory activity (measured by the capacity to reduce dinitrobenzene), as follows: kidneys, liver, brain and muscle. The hypothesis that in beri-beri there is a reduced oxidative capacity is thus supported. Hotta (1923) reports that the cholesterol content of nearly all body tissues increases during beri-beri. Only the kidneys and testes appear to be exceptions. Noteworthy changes were not observed in the total phosphorus content. Asada (1923) found the body fat in rats suffering from avitaminosis to be less than in normal rats. The diminution in cholesterol was not so great and the per cent of cholesterol in total fat therefore increases. The results are taken to indicate an increased transport of body and food fats to the seat of fat combustion in avitaminosis. Pappenheimer and Larimore (1923) found that lesions occurred in the esophageal portion of the stomach in rats fed faulty diets. These consisted of an inflammatory edema of the mucosa and submucosa with marked cellular reaction and in most cases with localized ulceration, associated with the inflammatory changes. There was always present more or less epithelial hyperplasia. These lesions were situated near the elevated ridge which divided the rumen from the glandular portion of the stomach.

Changes in Adrenals in Polyneuritis.—Beznak (1923) asserts that in polyneuritic pigeons the adrenals increased 2 to 3 times in size although the body weight and weight of the pancreas, thyroid, liver and stomach, decreased. The hypertrophy was absent in starved animals and in those receiving only vitamin B. Thus the hypertrophy is not due to hunger. The per cent of adrenalin in the adrenals was identical in both groups. In avitaminosis it is decreased. Gross (1923), contrary to the observations of McCarrison (1919), finds that absolute vitamin deficiency sets up very little alteration in adrenalin equilibrium, *i.e.*, relation between adrenalin store, gland weight and body weight in the adult rat, provided the rat is not brought to the point of death on the diet. Such alterations as do occur can be explained, he says, on a basis of general tissue atrophy. He believes that the striking changes reported by others have probably been produced by the accompanying starvation and unbalanced dietary rather than the deficiency in vitamins. It may be that the pigeon, monkey and guinea pig are predisposed to alterations to a much greater degree than is the adult rat.

Kato, Shizume and Maki (1921) stated that the nerve lesion resulting in paralysis in birds brought about by feeding polished rice is due to absorption of H-ions. Kato, Shizume and Kitasato (1921) observed that the propagation of the nerve impulse decreases greatly in nerves of fowls suffering with polyneuritis, and that finally there is a total loss of propagation of stimuli. This is attributed to H-ions, since the same effect could be brought about by H-ions when allowed to act upon a normal nerve. Recovery from paralysis upon injection of rice bran extract is so rapid that the disease cannot be considered due to histological nerve degeneration. They believe it is caused by a poisonous substance. The velocity of the nerve impulse measured in normal and in diseased nerves, and in nerves of cured fowls, confirmed their conclusions. A few of the nerve fibers remained impaired after the recovery of the fowl. The process of paralysis by the action of H-ions is by adsorption. Rice extract is very effective in restoring nerves impaired by H-ions but has no curative effect upon paralysis of nerves caused by urethane, chloral hydrate, etc. The sciatic nerve of fowls suffering from the disease had an H-ion concentration 5 to 10 times as great as the normal. That of the peripheral nerves was greater than that of the spinal cord. The H-ion concentration of the blood of

normal and diseased fowls was the same, but the power to neutralize acids was lessened in diseased fowls. Microscopic examinations showed the same degenerative changes in the myelin sheath in the axis cylinder in nerves paralyzed by H-ions as in the polished rice disease.

Miyadere (1921) found when vitamin-free test meals consisting of polished rice, pure corn gluten and lard were given to animals there was practically no gastric secretion. The diet was given until there was a marked disturbance. When 100 c.c. of a 5 per cent solution of 96 per cent alcohol was given a secretion was produced as great as before avitaminosis. He concludes that in dogs in avitaminosis the gastric secretion capacity is undisturbed since in the vitamin-free diet there was a complete lack of stimulants for secretion. Danyse-Michel and Koskowski (1922) fed pigeons as follows: (a) fed normally; (b) absolute fast; (c) fed polished rice only; (d) fed polished rice and given daily subcutaneous injections of 0.4 milligrams of histamin. At regular intervals after 7 days of feeding the gastric juice was obtained and examined with the following results: Birds from a, b and d secreted 6-7 c.c. of juice per hour. Birds from c secreted only 1 to 4 c.c. H-ion concentration of gastric juice was greatest in group d, slightly less in those of group b, much less in those of c. Peptic activity was positive but feeble in birds of group b, normal in d, and almost absent in those of c. An examination of the intestinal content of the birds showed that those of groups b, c and d during the first few days of the test were affected with intestinal disorder and hypersecretion of bile. The bacterial flora became much more abundant and active in c and d. It appears that pigeons fed polished rice are capable of secreting even at the time of paralytic crisis gastric juice not deficient in quantity, acidity and especially pepsin. Daily injections of histamin absolutely prevented nervous symptoms or paralytic crises in birds receiving a polished rice diet and these birds also secreted a constant and normal gastric juice. The death of pigeons fed polished rice is ascribed to the lack of plastic substance necessary for the evacuation of toxic products, to the lack of certain acid amines necessary to the reconstruction of tissues, and especially to the lack of enzymes.

Megaw and Banerji (1923) reported 12 cases of beri-beri which occurred in two families, well-to-do and highly cultured, consisting of 13 members, ranging in age from 8 to 80 years. The most striking manifestations of the disease were gastro-intestinal

symptoms which occurred in 10 out of 12, diarrhea in 8, and dysentery in 1.

Changes in Reproductive Organs Due to Lack of Vitamin B.—Novaro (1920) observed that pigeons fed a diet lacking in vitamin B exhibited degeneration of the tubules and hyperplasia of interstitial tissue in the testes.

Evans and Bishop (1922), in their studies on the existence of a vitamin concerned with reproduction, comment on the disturbance of estrous when vitamin B is omitted from the diet. They found that the animals not only failed in weight rapidly but in practically all cases cessation of estrous occurred at once. When vitamin B was added to the diet they found that the animals gained weight rapidly but did not have cycles for some time. Estrous is not prolonged in the characteristic way as was found to be the case in vitamin A deficiency, but is obliterated and the ovarian follicular apparatus comes to rest. They interpret these findings as demonstrating that ovulation is more sensitive to a lack of vitamin B than is general bodily nutrition.

Meyerstein (1922) states that the sex organs suffer especially in vitamin deficiency. The cells are poorly developed and fail in maturation. The interstitial tissue is relatively increased. Gotta (1923) fed pigeons a diet free from vitamin B and also with the addition of yeast. The testes and ovaries of the latter group were several times as large as those in the birds fed the vitamin-free diet, whose gonads were atrophied. Souba (1923) found that in cockerels fed a diet deficient in vitamin B there was a decrease in the size and weight of the organs in the following order: Testes, spleen, heart, liver, kidney, pancreas and thyroid. The testes were affected to the greatest extent. Baudette (1924) says that when hens were fed a ration poor in vitamins the number of fertilized eggs failing to hatch was larger than when the diet of the birds was normal and complete.

Changes in Blood in Avitaminosis.—Funk and Schönborn (1914) found an increased sugar content in the blood of pigeons to result from lack of the vitamin B and a diminished amount of glycogen in the liver. Hirai (1921) observed that a deficiency of vitamin B caused hyperglycemia in rats. Mathew (1921) stated that the addition of vitamin B to the diet of infants suffering from metabolic disturbances apparently increases the "index of tolerance" for food and results in a rapid increase in the appetite, weight and number of red blood cells. Abderhalden (1921) reported that pigeons fed polished rice have a red cell

count of not more than 50 per cent of the normal value. He did not note a loss either in total weight or in water content of the brain, heart, lung or kidney in starved pigeons or in pigeons fed polished rice with addition of yeast. Collazo (1922) found the blood sugar concentration in avitaminosis to be like that in partial starvation. He concludes that avitaminosis is a kind of under-nutrition. Palmer and Hoffman (1922) reported that the decrease in total solids and in the nitrogenous constitutents of the blood, both protein and non-protein, as well as the diminution in the erythrocytes are probably due to self-imposed starvation which accompanies the later stages of polyneuritis in pigeons. Simple inanition in its advanced stages is accompanied by a decline in the relative amount of protein in the blood which is not apparent even in acute polyneuritis.

Suski (1923) observed polyneuritic pigeons to show a low erythrocyte count. After 15 minutes' irradiation for 7 days the count was markedly increased. The leucocytes remained unchanged. Collazo and Bosch (1923) found that during the first 5 weeks on a vitamin-free diet dogs showed a slight hypo-lipemia. This was followed by a stage of hyper-lipemia which was at its maximum at 11 to 13 weeks. Thereafter blood fat decreased markedly, almost reaching the normal value at the end of the sixteenth week, when death occurred. Alpern (1923) stated that the amino-acid nitrogen which ran parallel with the rest-nitrogen increased in both groups. The alcohol-ether soluble fraction also showed a slight increase. Collazo (1923) reported the carbon dioxid combining power of the plasma to be lowered in the last stages of avitaminosis in dogs, guinea pigs and pigeons. This, he believed, not to be primary but the result of inanition.

Thompson and Carr (1923) found a marked increase in the uric acid and creatinin content of the blood with the onset of polyneuritis. Mattill (1923) stated that deprivation of vitamin B in contrast to fasting was not accompanied by an accumulation of nitrogenous products in the blood. Hayashi (1923) reports that the bicarbonate concentration of the plasma in beri-beri, excluding the cardiac form, varied from 0.692 to 0.31 per cent in 14 cases. The normal values were 0.708 to 0.535 per cent. The urea and nitrogen content of blood was slightly higher than normal. Nitzewcu and Cadariu (1923) noted that in pigeons fed either polished rice or autoclaved corn or a synthetic diet deficient only in vitamin B there was a diminution

of red cells and a lowering of the hemoglobin content. The resistance of the red cells also showed a tendency to diminish. There was a constant increase in the cholesterol content of the blood.

Infection and Vitamin B Deficiency.—Guerrine (1921) observed that pigeons whose vitality was lowered by restricting them to polished rice are no longer resistant to B. anthracis. The diarrhea of such pigeons is connected with a more strongly developed intestinal flora in which B. coli communis predominates. Well, Arloing and Dufort (1922) found that the administration of bacterial cultures of intestinal bacteria, either alive or dried at different temperatures, to pigeons which have been partly starved on a diet of polished rice had no effect on the progress of their bodily disintegration except in delaying the onset of death. Ishido (1922) reports that wound healing is delayed in guinea pigs and rats fed a faulty diet, *e.g.*, a cereal grain, unsupplemented or incompletely supplemented, and the susceptibility to infection is increased.

Relation of Avitaminosis to Tumor Growth.—Cramer (1923) stated the growth of cancer cells cannot be arrested by withholding from the host a supply of vitamins. With tryptophan deficiency the tumor grows, while the growth of the animal is arrested and some tissues even atrophy. Dietary restrictions of a degree to impair the health of the host do not arrest the growth of the tumor. McCarrison (1923) induced avian polyneuritis in pigeons and then cured them by a sufficient diet. Six to 12 weeks afterwards 7 of the 12 developed epithelioma contagiosum which was attributed to their poor nutrition during the initial experimental stage, producing an abnormal state of metabolism with disorder of endocrine function favorable to the entry into the body of a special virus fortuitously present. McCarrison (1923) reports a further outbreak of epithelioma contagiosum among pigeons fed a deficient diet. He says "the state of faulty nutrition favorable to the operation of the invisible virus of this malady is brought about by deficiency of certain substances in the diet, among which vitamin B is one."

Cramer (1920) described "Lipoid gland tissue" found in the white rat (1) at the back of the shoulders between the scapulae, (2) in the thorax around the aorta, (3) in the abdomen between the kidneys and the abdominal aorta and surrounding the adrenal glands which lie imbedded in it. In rats dead as a result of vitamin-free diets both fats and lipoids may be completely

lacking in this tissue. It is suggested that this lipoid gland tissue forms an important deposit of one or more of the vitamins. Cramer, Drew and Mottram (1921) report that the only demonstrable histological change in rats and mice in the final stage of avitaminosis is an extreme reduction of lymphocytes both in the lymphoid structures and in the blood. This condition of the lymphoid tissue is practically identical with that produced by exposure to X-rays and radium. These agents, if given in adequate doses, not only have the same effect on the animals—they lose weight and die in a state of emaciation—but degenerative changes in the seminiferous tubules of the testes, similar to those observed in avitaminosis, occur. It is suggested that probably the lymphoid tissue fulfills a more important function in connection with the nutrition of the animal than is generally recognized and that the effect produced on the general nutritive condition of the animals by these different agencies may be due to their selective action on the lymphoid tissues.

There exist differences of opinion as to whether or not beriberi is the result of vitamin B starvation alone, and the question cannot be decided on the basis of existing evidence. It also remains to be established whether or not the rat and the pigeon test for the anti-neuritic principle actually determine the same substance. There is still the possibility that what we call vitamin B consists of two or more substances, but no positive evidence has been brought forward which lends support to this view.

There are pathological changes which are specific for each of the deficiency diseases as they relate to vitamin starvation. It is not certain that the syndrome caused by deprivation for any one of the vitamins has been adequately described. In some of the experimental evidence presented in this chapter it is apparent that faults other than a lack of a vitamin existed, and it is possible that these contributed to the changes produced. The lesions produced by deficiency of different vitamins in the diet are so distinct that there seems to be no doubt that the manner in which each affects metabolism is peculiar to itself.

CHAPTER XIII

THE CHEMICAL NATURE OF THE ANTI-NEURITIC SUBSTANCE, VITAMIN B

Historical Survey.—Eijkman (1897) observed that a water extract of rice bran possessed anti-neuritic properties. He found the curative substance to be dialysable but not to be precipitated by the addition of alcohol. Grijns (1909) confirmed the experiments of Eijkman. He was the first worker to express the view that beri-beri developed when the diet was lacking certain substances which are of importance in the metabolism of the peripheral nervous system. Grijns found that the bean "katjang-hidjoe" (Phaseolus radiatus) also contained the substance which cured beri-beri (see page 203).

Fraser and Stanton (1909) examined various kinds of rice for their protection against beri-beri. Their studies led them to the belief that a rice poor in phosphorus would cause the disease. They suggested that the phosphorus content was a practical indicator of the nutritive value of rice. Schaumann (1908) suggested that beri-beri was a disturbance of metabolism due to the lack of organic phosphorus compounds.

Hulshoff-Pol (1910) showed that a watery extract of katjang-hidjoe beans clarified with lead acetate contained the curative substance. He obtained a crystalline substance which he called X-acid. Eijkman (1911) found that the active substance of yeast could be extracted with 88 per cent alcohol. Teruuchi (1910) extracted rice bran with dilute hydrochloric acid, neutralized the solution, evaporated the filtrate and extracted the residue with alcohol. This extract he found to be active and to contain only a small proportion of its original phosphorus content. Similar results were obtained by Chamberlain and Vedder (1911).

Funk (1922) summarized the knowledge available of the chemical nature of the anti-neuritic vitamin up to 1911 as follows: (1) The substance is soluble in water, alcohol and acidified alcohol; (2) it is dialysable; (3) it is destroyed at 130° C.

Attempts to Isolate Vitamin B.—Funk (1911, 1912, 1913) made the first elaborate effort to isolate and identify the anti-beri-beri vitamin. He found that although alcohol extracts a part of the physiologically active substance from rice polishings, water is far more effective. He found it to be precipitated by phosphotungstic acid or by silver nitrate and barium hydroxid, and partially by mercuric chlorid. Funk stated that the curative substance contains nitrogen and is free from phosphorus. He suggested that it was a pyrimidin.

Schaumann (1910) attempted to identify the substance in rice polishings which cures polyneuritis. Polished rice has a much lower phosphorus content than unpolished. The polishings which are rich in this element are also highly effective in curing the disease. These facts led him to suggest that the substance under investigation was akin to nucleic acid. Nucleic acid occurs in the nuclei of cells. It is composed of four organic bases, four molecules of sugar and four molecules of phosphoric acid. He abandoned this view in 1912 when he isolated from rice bran a phosphorus-free substance which would cure polyneuritis in pigeons.

Suzuki, Shimamura and Odake (1912) prepared "oryzanin" from rice polishings. This was certainly not a pure compound. They have prepared from this a highly active picrate, which, however, appears to contain at least two substances. In the present state of their work it is stated that both flaky and needle-like forms of the picrate occur. The needlelike form is much less active, and it seems probable that the needles obtained by these investigators owed their activity to an admixture of the flaky crystals, since these would not have been removed by their procedure.

Edie, Evans, Moore, Simpson and Webster (1912) obtained an active fraction from yeast, which they regarded as a pyrimidin. This they termed torulin. Funk (1912) showed that a substance which was organic and basic could be isolated from rice bran by extraction with alcohol containing hydrochloric acid. It was precipitable by phosphotungstic acid, silver nitrate and barium hydroxid, and partially by mercuric chlorid. It cured polyneuritis induced in birds by feeding polished rice. He named the substance "vitamine" (1913-1914). Chamberlain and Vedder (1911), and Edie, Evans, Moore, Simpson and Webster (1912) made similar observations.

Funk (1913) isolated nicotinic acid from rice polishings. It

was suggested that this or some unstable modification of it is the anti-neuritic substance. Extracts of rice polishings may develop a pyridin-like odor. Drummond and Funk (1914) noted that preparations capable of curing pigeons gave a blue color with the Folin-Macallum phenol reagent, indicating the presence of hydroxyl groups (1912-1913). McCollum and Kennedy (1916) found that any extracts of natural foods which furnish vitamin B in growth tests with rats effected "cures" of polyneuritic pigeons. They demonstrated that vitamin A plays no rôle in the production of polyneuritis in pigeons. They also found that acetone, ethyl acetate and benzene can extract some substance from ether-extracted wheat germ in sufficient amount to cure pigeons but which gave negative tests for growth in rats.

Williams, and Williams and Seidell (1916) prepared adenin and certain α-hydroxy pyridins which had anti-neuritic properties. These preparations lost within a few days all curative power. They drew the deduction that the substances changed from an unstable to a stable form and that the latter was inactive. They treated the inactive preparations with alcoholic sodium hydroxid and reported that this transformed them into the active form. They believed that they had in the active preparation the enol form of the substance. None of their synthetic preparations had the activity or certainty of action seen in extracts of natural foods. Williams suggested that polyneuritis is not due to a deficiency of a substance *per se,* but to a lack of a certain type of potential energy which only certain substances can supply. He suggested that it was the potentiality of isomeric change in the substance administered which restored a paralyzed bird. To this isomeric change Williams attributed the instability of the vitamins of natural foods. He did not assert that the anti-neuritic vitamin is a hydroxy-pyridin, but suggested that the same type of isomerism is possible in bodies of entirely unrelated natures such as pyrimidins, purins and other heterocyclic compounds.

Williams and Seidell (1916) absorbed by fuller's earth the anti-neuritic substance from autolyzed yeast. They extracted the active material from the earth with 5 per cent sodium hydroxid and by barium hydroxid. The extracted material yielded crystals which were found to be inactive and were identified as adenin. When adenin was treated with boiling glacial acetic acid, acetic anhydrid, dilute hydrochloric acid or sulphuric acid, or allowed to stand for some time in the presence of dilute

mineral acids or heated to boiling with concentrated sulphuric acid, it was reported to have acquired anti-neuritic properties. They stated "an isomer of adenin is the chemical entity responsible for the characteristic physiological properties of the vitamin under investigation." They regarded their work as "of chief interest not in its contribution toward establishing the composition or identity of this 'vitamine,' which may or may not be a unique compound, but in affording corroboration of the theory advanced that isomerism plays a determinative rôle in the physiological potency of 'vitamines.'"

Voegtlin and White (1916) and Harden and Zilva (1917) have attempted unsuccessfully to repeat the studies of Williams and Seidell purporting to show that adenin and α-hydroxy pyridin can acquire anti-neuritic properties. McCollum and Koch (unpublished data) attempted to induce growth in young rats on diets lacking the anti-neuritic substance, but containing adenin, pilocarpin hydrochlorid or adrenalin. In no instance were they successful. McCollum and Simmonds (unpublished data) tested thymus nucleic acid as a source of vitamin B for rats, but with negative results. The behavior of the polyneuritic pigeons led to the acceptance of the view that the lesions in this disease were principally situated in the nervous system.

Williams (1917) again expressed the view that under certain conditions α-hydroxy pyridin may possess anti-neuritic properties. He attributed these properties to the existence of this compound in the form of a pseudo-betain, and suggested that a configuration conforming more or less closely to that of the betain ring was probably an essential characteristic of the anti-neuritic vitamin. It was pointed out that such a structure was possible in most of the simpler nitrogenous components of animal tissues, especially in the purin bases.

Abderhalden and Schaumann (1918) attempted to isolate the anti-neuritic substance from yeast. They introduced the terms eutonin and nutramin to designate certain vitamin-rich preparations. Hofmeister (1918) isolated a substance which he regarded as a pyrimidin, which possessed anti-neuritic properties.

Attempts to Concentrate Vitamin B.—Osborne and Wakeman (1919) described a method for preparing an extremely potent preparation of vitamin B. They prevented disintegration of yeast cells by boiling in acidified water after thorough washing. The protein was then coagulated and an extract free from the products of autolysis was obtained. The extracts from

4½ kilos of fresh yeast extracted with 15 kilos of water were concentrated to two liters and poured into three liters of 93 per cent alcohol. The precipitate which formed was inactive. The filtrate was evaporated to 300 c.c. and poured into 1960 c.c. of 93 per cent alcohol. The second precipitate was dissolved twice in water and precipitated with alcohol so that the alcohol content was 90 per cent. This precipitate amounted to 6.2 per cent of the dry yeast and contained almost all the vitamin B present in the yeast. The preparation was acid in reaction and gave a precipitate with lead acetate and with mercuric chloride. Twenty-five per cent of the substance was precipitated with silver nitrate and barium hydroxid.

Levene and van der Hoeven (1924) have prepared an extraordinarily potent preparation of vitamin B. They concentrated the substance by the method of Osborne and Wakeman, then adsorbed it on silica jel. From this it can be extracted by alkalies and also by acids of pH 3. They found that barium hydroxid precipitates the vitamin from its solutions but incompletely, but a very potent preparation was secured (200 to 400 times as potent as that of Osborne and Wakeman). They found the following procedure a good one for the separation of the vitamin in highly concentrated form. The original barium precipitate is suspended in water through which a stream of carbonic acid is passed. The principle underlying this method is the following. In the barium precipitate the substances with acidic properties stronger than carbonic acid remain undissociated and stay in the solid phase, whereas the substances with the opposite properties pass into solution. A priori, it was not possible to predict in which fractions the active material would be found. Experience showed that by this process most of the inert material passed into solution, whereas the potent material is contained in the insoluble part. When the latter is freed from barium the material is active in about 0.00017 gm. of nitrogen per day. This material is obtained with a very little loss of vitamin and serves well to concentrate the growth promoting principle contained in yeast.

Myers and Voegtlin (1920) attempted the chemical isolation of vitamin B by adsorption. Mastic, Lloyd's reagent and iron sulphate were unsatisfactory because they lacked specificity. Olive oil and oleic acid removed the anti-neuritic substance from autolyzed yeast filtrate. Stachydrin, trigonelline and allied betains showed no anti-neuritic activity. Histidin and its

esters were likewise inactive. The active principle is readily extracted from dried yeast by means of acid methyl alcohol.

Seidell (1921) described a method for the preparation of a relatively stable silver compound of the anti-neuritic substance. He discovered that fuller's earth has the property of adsorbing from its solutions the physiologically active substance. From the fuller's earth the vitamin is extracted incompletely by barium hydroxid. The barium is quickly removed from the extract as sulphate and lead acetate is subsequently added as a precipitant. The excess of lead is removed as sulphid, and the filtrate from this precipitate is evaporated rapidly under diminished pressure to a small volume, when a white amorphous substance separates. This is inactive and is discarded. Evaporation is continued in a vacuum desiccator and several crops of the white substance are removed. The solution is evaporated to a thick viscous mass which can be brought to dryness. The physiological activity of the dried residue is retained for many months. When this material is dissolved in a small volume of water and treated with silver nitrate a voluminous precipitate is formed which is not anti-neuritic. This precipitate is removed. To the filtrate silver nitrate and ammonium hydroxid are added. A second silver precipitate is formed which contains the vitamin. The filtrate is practically free from it. This procedure is a distinct step in advance in the purification of this most interesting principle.

Differential Dialysis of Vitamin B.—Zilva and Miura (1921) reported that in differential dialysis of the anti-neuritic factor they used autolyzed yeast as a source of the vitamin. Vitamin B failed to diffuse appreciably through a membrane which had been soaked in 90 per cent alcohol (90 per cent membrane). It did entirely diffuse through a 95 per cent membrane. The anti-neuritic factor diffuses through a collodion membrane of such permeability as to permit the passage of substances like methylene blue, neutral red and safranin. It is suggested that the active molecules, whether simple or associated, may be of a semi-colloidal state.

Tsukie (1922) reported the isolation of vitamin B in the histidin fraction with silver nitrate and barium hydroxid, from the phosphotungstic acid precipitate of an alcoholic extract of bran, following the usual procedure for separating nitrogenous bases. This vitamin B preparation was a brown-yellow powder, easily soluble in water and acidified alcohol. Aqueous solutions

of the substance gave no reactions for proteins or amino-acids, or any of the following: Millon's, Weidel's, Abderhalden-Schmidt's, biuret, xanthin, murexid, and diazo reaction.

Marchlewski and Wierzchowski (1922) report a method for concentrating vitamin B from wheat bran. They extracted with .1 per cent hydrochloric acid and neutralized the extract with ammonium hydroxid. The precipitate formed was inactive and was removed. The solution was precipitated with mercuric nitrate in acid solution. The precipitate was decomposed with hydrogen sulphid and the filtrate evaporated to dryness. The residue was inactive, showing that the mercury did not precipitate the vitamin. Another portion of the original filtrate from the precipitate formed on adding ammonium hydroxid was treated with an ammoniacal solution of silver nitrate. The precipitate was dissolved in hydrochloric acid, the solution evaporated to dryness and the residue dissolved in water. This solution was very active, while the filtrate from the silver precipitate was inactive. This shows that silver precipitates the vitamin. The active solution on neutralization with sodium hydroxid gave an inactive precipitate and an active filtrate. On the addition of picric acid to this filtrate small prismatic crystals separate which had curative properties. They had no definite melting point, but decomposed between 180 and 200°. The same authors (1924) state that vitamin B is extracted from brewer's yeast better by acidified water than by ethyl alcohol. Mercuric nitrate precipitates the vitamin but slightly. Silver nitrate gives a slight green-brown precipitate. This treated with hydrochloric acid gives a brown-yellow solution, which evaporated to dryness at 40° in vacuo, and redissolved in water, is very active. This solution neutralized with sodium hydroxid, filtered and precipitated with picric acid, gives a yellow orange precipitate of minute rods which can be recrystallized from acetone. The picrate was decomposed by hydrochloric acid and the picric acid taken out with ether. The solution was neutralized and the resulting brown-yellow solution had the same marked curative effect as the picrate. From 1 kilo of yeast .022 of a per cent of vitamin picric was obtained.

Damianovich (1922) reports that extracts of brewer's yeast, although almost colorless, showed a characteristic absorption band between 2,478 and 2,660 A°, which seemed to correspond to the pyrimidin compounds. Bertrand and Seidell (1923) described an analysis of the mineral elements in their extract

containing the anti-neuritic substance, and also the method for the preparation of a picrate, 2 mg. of which protected a pigeon against polyneuritis. Recrystallization with water gave 2 crystal forms, one of needles which had a melting point of 202° C., the other consisting of crystalline particles which were very irregular and had a melting point of 160°.

Studies on Vitamin B.—Seidell (1923) described the preparation of a silver compound of the anti-neuritic substance from yeast. The preparation is quite stable both in solution and when dry. It dialyzed almost completely through collodion, all the vitamin being found in the diffusiate. This suggests that the molecule is relatively simple. Seidell believes that the vitamin in his preparations is still contaminated by one or more nitrogenous bases, which cannot be advantageously separated by precipitation with silver. Seidell (1924) further discussed the preparation of the picrate of the vitamin B. Of his preparation as little as 2 mg. daily protected pigeons from loss of weight on a polished rice diet. He obtained both rod and needlelike crystals in his picrate which were but little active, and he suggests that the needles obtained by Suzuki, Shimamura and Odake owed their activity to an admixture of a small amount of the vitamin itself, since the method which they describe would not have effected a complete separation of the substances.

Pigeon and Rat as Test Animals for Vitamin B.—The procedure of Funk has been almost universally followed to determine the relative values of different food-stuffs, or of preparations made with the object of isolating and identifying the anti-neuritic substance. This consists in feeding pigeons polished rice until acute symptoms of neuritis appear. The substance to be tested is then given and the recovery of the bird's powers of locomotion interpreted as a positive test. Failure of the bird to respond to this treatment is accepted as a negative test for the vitamin.

It was pointed out by McCollum and Simmonds (1918) that this test is uncertain. At that time they accepted the prevailing view that nerve degeneration was the most pronounced lesion in avian polyneuritis, and emphasized the probability that substances of a stimulating nature might whip up motor nerve cells which had lost their functional capacity, and temporarily restore the birds to an apparently normal condition. They pointed out the high degree of improbability that so many totally unrelated chemical substances as pyrimidins, purins and pyridins could fulfill the same physiological rôle. They were led to suspect that

the "curative" substance was not necessarily identical with the indispensable nutritive principle essential for growth and normal functioning, but rather a body which possessed the pharmacological property of goading to renewed activity nerve cells which had been injured and had ceased to functionate. The observations of Dutcher (1919) that desiccated thyroid gland, thyroxin, pilocarpin hydrochlorid and other physiological stimulants are able to induce "cures" of pigeons in polyneuritis, supports this view.

Williams (1921) reports curative effects by administration of α-hydroxy pyridin to polyneuritic birds. α-methyl pyridon, trimethyl uracil and 4-phenyl-iso-cytocin, gave slight protection. He considers vitamin B to be a cyclic nitrogen compound with a substitution of oxygen in the ring and capable of existing in a betain configuration. Funk (quoted from van der Walle, 1922) says that the recovery of pigeons by treatment with hydantoin lasted only 9 days, whereas a pigeon treated with thymus nucleic acid lived for a fortnight. Allantoin, adenin and other pyrimidin derivatives were less active. Uric acid was ineffective. Takahashi (1920) says cholesterol, lecithin, kephalin and protagon do not contain vitamin B, although the last gives temporary relief in polyneuritic pigeons. Mattei (1923) says pigeons in an advanced stage of polyneuritis induced by polished rice showed marked recovery by treatment with an infusion of roasted coffee. Raw coffee and pure caffein were without effect. Theiler (cited by van der Walle) reports favorable results with polyneuritic pigeons by subcutaneous injections of distilled water. The findings of McCarrison that the nervous symptoms in avian beri-beri are of secondary importance, further emphasizes the rationality of the view that the pigeon test, as ordinarily carried out, is without value for the specific purpose for which it has been so widely employed.

McCollum and Simmonds described a procedure for determining the presence or absence of the vitamin B. The procedure is to restrict young rats to a diet of purified protein, dextrin, a salt mixture and butter fat or other fat which contains vitamin A. On such a diet the animals are unable to grow after being confined to the diet for 3 or 4 weeks and ultimately die with or without the development of symptoms of polyneuritis. After 3 to 5 weeks there is added to the diet a substance to be tested. If the animals recover and *resume growth,* the test is positive. If they continue to decline the substance tested for is absent, or present

in amounts too small to meet their nutritive needs. This test requires considerable amounts of material. Only after a week or two is the outcome of the test apparent. There is no chemical test yet discovered which in any way assists us in tracing the vitamin B in attempts to isolate it. Funk (1913) called attention to the fact that his active preparations (tested on pigeons) never failed to give with the Folin-Macallum reagent a test for the phenol group. This reaction is given by proteins, and by a large number of organic compounds regularly met with in biological work.

Proposed Test for Vitamin B.—Jendrassik (1923) sought to find a qualitative test for the vitamin B. His method was to extract rice polishings, wheat germ, navy bean, spinach and carrots among foods which contain the vitamin B in fairly liberal amounts, and polished rice, white flour and beefsteak among foods containing little or no vitamin B, with solvents which do and which do not extract the vitamin. He used 50 per cent alcohol, absolute alcohol, ether, acetone and benzene. In some experiments rice polishings, wheat germ and navy beans had been boiled with 5 per cent sodium hydroxid to destroy the vitamin B before extraction. This yielded a large number of extracts which contained various extractives removed from the natural foods by means of the different solvents, and it was clear from experimental tests on rats which of these contained sufficient vitamin B to induce growth in the animals. With these extracts from several different kinds of food-stuffs it was hoped that the vitamin would not be contaminated in the case of different food extracts with the same list of impurities and that where the extracts were made from vitamin-free foods the contaminating substances would be present and serve to avoid error in deciding on the interpretation of any color reactions which might be applied. Jendrassik tested many reagents on this series of extracts and found certain chemical reactions were common to all the preparations containing the vitamin B, but were not given by the preparations not containing it. He proposed a reduction of ferric ferricyanid with the formation of a blue color as a test for the vitamin B. In his series this reaction was not given in a single instance by an extract of food containing vitamin B when the solvent used was one which does not extract that substance. The test was in no instance given by an extract of a food which does not contain the vitamin B although the solvents used extract this principle when present. Such solvents are water,

dilute alcohol and glacial acetic acid. Phenols, amino-acids and most alkaloids were, he believed, excluded as substances giving the reaction for this vitamin.

V. E. Levine (1924, personal communication) made a critical study of Jendrassik's reagent and concludes that the ferric ferricyanid reaction is not a specific test for vitamin B. It is given by phenols and may serve as a test for phenols in the absence of a few positively reacting non-phenolic compounds. Alkali reacts with phenols to give derivatives or fragments that no longer respond to the ordinary phenol tests. In respect to alkali treatment vitamin B responds in a similar manner. The finding that active preparations of vitamin B do not give a positive Millon or Liebermann is no proof of the fact that vitamin B is not a phenol, since neither the Millon nor the Liebermann reaction is given by all phenols. Levine's studies do not exclude the possibility of the presence in the vitamin B molecule of one or more phenolic group.

Bezssonov (1924) says Jendrassik's results do not justify his affirmation of the non-existence of the phenol function in vitamin B. The reduction of the reagent is a very sensitive reaction for o- and p-polyphenols.

Solubility of Vitamin B.—Jansen (1920) says 0.3 per cent hydrochloric acid, 70 per cent and 96 per cent alcohol containing hydrochloric acid were used to extract rice bran. The extracts were given to cocks and doves. The vitamin is quantitatively extracted and different methods gave the same results. Wells (1921) describes the manufacture of tiki-tiki extract made by the application of 25 per cent alcohol to rice polishings. This preparation is distributed gratis to mothers in the Philippine Islands who have infants suffering from beri-beri, and to nursing mothers. Eddy (1921) reported that glacial acetic acid was a solvent for vitamin B. Levine, McCollum and Simmonds (1922) confirmed this observation.

Funk, Harrow and Paton (1923) studied the comparative values of the following solvents in extracting vitamin B from yeast: ethyl alcohol (50, 60, 70 and 80 per cent), methyl alcohol (60 to 70 per cent), propyl alcohol (70 per cent), butyl alcohol (70 per cent), isobutyl alcohol (70 per cent), acetone (70 per cent), methyl ethyl ketone (70 per cent) and acetic acid (70 per cent). The extracts and residues were tested on pigeons and rats, and the extracts were also tested for their content of bios, co-ferment, total nitrogen and total solids. If inactivity of

residues be taken as a criterion, then 70 per cent alcohol has proved to be the best solvent for the anti-neuritic vitamin among those tested. Vitamin B and bios do not run parallel, so that as a rule the higher the content of vitamin B used the lower is that of bios. Using rice polishings instead of yeast 60 per cent alcohol was better than 70 per cent. The co-ferment showed no definite relation either to vitamin B or bios. The extracts from rice polishings seemed to be particularly active when tested on rats, and by comparison far less so when tested on pigeons.

Destruction of Vitamin B.—The anti-neuritic substance is relatively stable toward acids, but loses its physiological value when treated with alkalies. Vedder (1913) reported that strong alkalies such as sodium hydroxid, ammonium and barium hydroxid destroy the neuritis-preventing vitamin. Sullivan and Voegtlin (1916) observed this destructive effect of alkalies, and their observations have been confirmed by McCollum and Simmonds (1918), and by Osborne and Leavenworth (1921). Zilva (1922) stated that vitamin B is not destroyed by ozone, at least to the extent that vitamin A is, but vitamin C is destroyed. Williams (1924) reported that the anti-neuritic factor in an aqueous extract of yeast was destroyed completely by irradiation for a few hours when exposed to the rays of a quartz mercury arc lamp in a layer 2-3 mm. deep.

Osborne and Mendel (1922) made an interesting study of the quantitative aspects of the vitamin B requirement of rats. Rats of different ages, previously maintained on a normal mixed diet, were placed on a diet of casein 18, starch 54, lard 15, butter fat, and salt mixture 4 per cent, with varying additions of dry yeast. When the rats had reached a weight of approximately 40, 70,150 and 240 gm., the addition of 200 mg. of dry yeast daily was adequate for all; 100 mg. was less satisfactory, particularly for rats weighing more than 200 gm.; 50 mg. was still less satisfactory, and 25 mg. failed to secure even maintenance of weight in the larger animals. The calculations indicate that the daily requirement of that particular yeast was from 50 to 60 mg. per 100 gm. of rat. Similar results were obtained with the yeast concentrate.

Vitamin B Content of Liver.—Steenbock, Sell and Jones (1923) found that the vitamin B content of the liver of rats did not vary greatly with the amount of vitamin B in the food. They fed various amounts of vitamin B to their stock animals, but even rats of different ages when put on a ration with little or no vitamin B all maintained life for about the same length

of time. Within certain limits the older and larger the rats the greater the loss in weight before they die. They believe that the rat has little ability to store vitamin B. Steenbock, Sell and Nelson (1923) emphasized the point of keeping rats on screens when tests are being made of the vitamin B content of foods. They say that when rats are prevented from consuming their excreta (urine and feces) the vitamin B content of the experimental ration must be at least twice as high to promote growth. They state that their rats were fed according to the group system and were confined in a rather large cage measuring two feet square by twenty inches high. They say "With such a large floor space the cages do not become filthy rapidly and the bedding remains dry. For this reason they were not cleaned oftener than at intervals of several weeks and excreta for consumption were generally available." It is probably to be expected that rats kept in filthy cages, with the feces from normal rations available for consumption, after the rats have suffered from the effects of a deficient diet, would influence the results. It remains to be determined how far eating of feces influences the results in quantitative vitamin studies under the conditions of good laboratory management. It is difficult to accurately appraise the data from laboratories where the animal cages are allowed to accumulate filth. We are aware that such a condition is all too prevalent in nutritional research laboratories.

Osborne and Mendel (1923) report that when rats are fed a diet low in vitamin B their livers are found not to be rich in this substance. They determined this by feeding livers from rats on a low vitamin B diet to other rats confined to a diet free from this substance. The latter rats grew poorly, since these livers were such poor sources of vitamin B.

Vitamin B in Urine.—Muckenfuss (1918) says the antineuritic vitamin is probably present in small amounts in fresh filtered bile from the bladder of the ox. It is probably also present in filtered fresh human urine. Van der Walle (1922) stated that concentrated normal urine cures polyneuritis in pigeons. Urine of a dog has strongly curative effect, as has the urine of man. When the dog was fed a vitamin-free diet the urine lost its anti-beri-beri value. He was unable to detect any anti-scorbutic value in urine.

Vitamin B Content of Foods.—Dunham (1921) tested upon rats the relative vitamin B content of several vegetable foods. He found the daily dosage in milligrams of food necessary to

prevent loss of weight to be as follows: Baker's yeast 50, raw potato 65, potatoes boiled with skins 95, potatoes boiled without skins 125, potatoes baked 100, spinach boiled 85, cabbage raw 70, cabbage boiled 95, carrots raw 70, carrots boiled 105, turnips boiled 105, peas canned and uncooked 115, peas canned, boiled, 125. A comparison of the raw and cooked foods shows that some of the vitamin is extracted by the cooking and is lost in the water.

Orton, McCollum and Simmonds (1922) tested onion root hairs and found them to contain vitamin B. This warrants the belief that the vitamin is not concerned in the structure of the chloroplast. The mushroom (*Agaricus campestris*) proved to be a good source of vitamin B. Indian pipe (*Monotropa uniflora*), a non-chlorophyl bearing plant, gave results which were inconclusive, while the parasitic plant, dodder (*Cuscuta gronovii*), proved toxic and caused the death of the animals.

Corda (1923) reports that the appearance of beri-beri is delayed 50 days if pigeons fed polished rice are given 2 gm. of dried asparagus daily. Pigeons fed polished rice supplemented by asparagus lived 28 days longer after injection with B. anthrax than when the asparagus was left out. When they were fed the asparagus alone the resistance was not increased. He believes this to indicate that the vitamin B in asparagus was not alone responsible for the increased resistance.

Destruction of Vitamin B by Heat.—Johnston and Hooper (1921) reported that pigeons fed mixtures of spray process skim milk powder with polished rice require 30 per cent of the food in skim milk powder to prevent polyneuritis. This is equivalent to 75 c.c. of liquid milk daily. Johnston (1921) says drying skim milk by the spray process does not injure the vitamin B. He says it takes 2.5 parts of dry milk to 1 of purified food-stuffs to insure normal growth. Excessive amounts of milk caused subnormal growth after a time. Nitzescu (1923) observed that heating grains at 100° for 1 hour destroys the vitamin B. Heating at 120° for an hour and a half in an oven has apparently no injurious effect. He believes the destruction in the autoclave to be due to the superheated steam.

Sherman and Spohn (1923) studied the problem of the refinement which can be secured in rat growth experiments where the objective is to determine as nearly quantitatively as possible the content of vitamin B in a food. They conclude that a decrease of certainly 25 per cent, and probably 15 per cent, in the content of vitamin B can be detected. The quantities of milk selected as

the most advantageous to feed in order to detect possible reduction in vitamin B on heating were 0.8 gm. of dry or 8 c.c. of fluid skim milk per rat per day when fed separately from the basal ration, or 25 per cent of the total food mixture when the milk powder was fed with the basal diet. There was no evidence of any measurable diminution in milk powder content of vitamin B heated dry with free access of air at 100° for even 48 hours. When it was heated in the fluid state for 6 hours at 100° there was an important diminution of about 25 per cent in its content of vitamin B. Sherman and Grose (1923) studied the effect of 4 hours heating at 100, 110, 120 and 130 degrees upon the vitamin B content of tomato juice by the rat growth method. The tomato juice has a pH value of 4.3. A dosage of the juice giving approximately maintenance of weight during the 8 weeks' experimental period was found to be the best method of feeding; the disturbing factors were thus minimized and the sensitivity of this method is approximately at a maximum. The average figure for the destruction of the vitamin B obtained directly by determining the size of heated doses at each temperature necessary to give weight curves approximately coinciding with those of positive controls fed 4 c.c. of unheated juice, are 20, 33, 47 and 55 per cent at 100, 110, 120 and 130 degrees respectively. There is no indication of an increased temperature coefficient of destruction around 120°, although as with vitamin C the temperature coefficient (1.3-1.4 per 10°) is smaller than that of the average chemical reaction.

Vitamin B Requirement of Different Species.—Emmett and Allen (1919) found that a large amount of fat in the diet was very injurious both to the growth and the development of the hind legs in tadpoles. Apparently the lower percentage of fat the better the tadpoles thrive. Vitamins A and B appear to be necessary for their growth and development. Emmett, Allen and Sturtevant (1920) report that for complete metamorphosis iodin in the inorganic or organic form, as in desiccated thyroid gland, is essential. Harden and Zilva (1920) confirm the observations of others that vitamin B is essential in the diet of the frog. Bacot and Harden (1922) confirm Northrup's views that drosophila require vitamin B. They were not certain as to the requirements of the fly for A and C.

Plimmer and Rosedale (1922) report that pigeons require the smallest amount of vitamin B of any birds studied. Chickens require more than pigeons. Ducks, geese, guinea fowls, turkeys

and pheasants need more than chickens or pigeons. They suggest that leg weakness may be due to an insufficiency of vitamin B in the diet, since they found chicks were very susceptible to an insufficiency of vitamin B. They interpret their results to indicate that the amount of vitamin B necessary runs parallel to the amount of carbohydrate and fat supplied the chicks. Hart and co-workers demonstrated that vitamin D and irradiation play a rôle in preventing leg weakness. Laufberger (1923) found that fish restricted to diets deficient in vitamins lost 4 per cent in weight or died before the termination of the experiment. Those receiving vitamins A and B gained 17 per cent in weight. Those getting only vitamin B gained 13 per cent. The controls getting A, B and C gained 19 per cent.

Although vitamin B has been known longer than any other member of the group, its nature is still unknown. Considerable confusion in the interpretation of existing experimental data results from the use of the pigeon by some and the rat by others as a test animal in the study of preparations assumed to contain vitamin B. Doubtless the entire subject will be clarified within the next few years.

CHAPTER XIV

BIOS AND THE GROWTH OF YEAST

Investigators are very desirous of isolating and identifying the vitamin B with a view to its possible synthesis. They have been anxious to compare in a quantitative way the amounts of this substance in different foods. The earliest efforts in this direction were made by attempting to find the smallest dose of a food, or extract of a food, which would induce a "cure" of a pigeon in polyneuritis as a result of restricting the bird to a diet of polished rice. This requires a series of tests and consumes a considerable amount of material. Another test consists of including a certain amount of the substance to be tested in the diet of a rat which provides no other source of the vitamin. This, like the pigeon test, requires considerable material. The amount of vitamin B in any food is apparently very small and its separation laborious and costly. It is, therefore, highly desirable that some test be devised for estimating this substance which is more economical both of time and material and yet gives highly accurate results.

Yeast as Test Organism for Vitamin B.—Williams (1919) proposed such a test for the vitamin B. He used as a culture medium a solution of inorganic salts and cane sugar. In this the growth of yeast was found to be very slow and unsatisfactory. The addition of unknown substances which can be extracted from natural foods by means of water and dilute alcohol greatly stimulated the proliferation of the yeast. In hanging drop cultures the number of progeny of a single yeast cell, counted after an interval of a few hours, constituted the basis of judgment as to the content of the solution in the vitamin. This test appeared for a time to be a great step in advance. since it promised to detect very small amounts of the vitamin in a short space of time and with a minimum requirement of material.

Bachmann (1919), in studying the vitamin requirements of yeast, found that all of the yeasts which she used grew better

and fermented more readily in a medium containing organic material extractable from natural foods. She also found that some yeasts would grow in and ferment a solution of inorganic salts and sugar even when introduced in small amounts. Bachmann states that Pasteur (1871) had already recorded this observation. She isolated a strain of yeast from canned pears which would not grow in or ferment a solution of inorganic salts and sugar unless large seedings of yeast were introduced, and even then fermentation was feeble. For this yeast some organic substance not contained in Nägeli's solution had to be added to stimulate budding. When substances especially rich in vitamin B were added to the solution fermentation resulted. She stated: "My work suggests that such yeasts as this one might be used to determine the presence of certain vitamins and possibly also that they might be used to determine quantitatively the amount of vitamins present in various food substances."

Williams (1920) extended his investigations to the development of a gravimetric method, in which larger amounts of yeast were grown than in the hanging drop method. About 0.3 mg. of yeast was used for seeding and the harvest was collected after 18 hours on a Gooch crucible and weighed. He (1921) found that baker's yeast tested by the yeast growth method showed a much higher vitamin content than brewer's yeast, whereas by animal experiments brewer's yeast was the richest.

Emmett and Stockholm (1920) discussed the relation of the anti-neuritic and water-soluble B vitamins to the yeast growth-promoting stimulus. The rate of growth of yeast was accelerated by the addition to the synthetic media of very small amounts of preparations which they had shown by biological tests to contain the anti-neuritic and water-soluble vitamins (vitamin B). They concluded that the yeast growth-promoting factor does not appear to be the same as the anti-neuritic or anti-beri-beri vitamin (pigeon). The yeast growth stimulus, they believe, is different from the water-soluble, growth-promoting vitamin (with rats).

Yeast Growth Not Satisfactory Test for Vitamin B Studies.—Souza and McCollum (1920) attempted to use yeast cell multiplication as a test for vitamin B and showed that no confidence could be placed in it. Yeast grows slowly in a vitamin-free medium. They used a solution of inorganic salts supplying nitrogen as ammonium sulphate and energy as cane sugar. They found that yeast grows slowly in this medium and that the addition of extracts of natural foods which had been so treated as to

destroy the vitamin B was as effective in stimulating the multiplication of yeast as were other extracts containing the vitamin.

Funk and Dubin (1920) described another test for the anti-beri-beri vitamin by the yeast growth method. They developed a quantitative method of measuring the yeast by collecting the harvest in capillary tubes by means of a centrifuge. Swoboda (1920) modified the Williams test for vitamin B by using yeast and tested various extracts of animal organs. Miller (1920) using Swoboda's yeast method studied the vitamin B content of vegetables. Whipple (1920) found the substance in onions and cabbage which accelerates the growth of yeast is not destroyed by heating to 100° for 30 or 60 minutes, nor by heating with acid (5 c.c. of vinegar to 100 c.c. of medium), nor with alkali (1.1 per cent of sodium bicarbonate).

Fulmer, Nelson and Sherwood (1921) discussed several mediums for the growth of yeast. They state that the optimum concentration of ammonium chlorid for the growth of yeast is that concentration of the salt in which a protein (wheat gluten) is least swollen. The optimum concentration of ammonium chlorid varies with the temperature. At the temperatures studied the concentration of the salt causing the least swelling of the protein is identical with those most favorable for the growth of yeast. They further state (1921) that treatment with alkali does not impair extracts of wheat germ or of alfalfa as yeast growth stimulants. Evidently the stimulant is not vitamin B. Extracts of alfalfa and of wheat embryo contain sufficient nitrogenous and inorganic material for the growth of yeast. A medium of known constituents was developed which promoted the multiplication of yeast without the addition of vitamins. They state that the addition of vitamin B did not improve this medium. The reason for this statement is that in their medium F, which is composed of known constituents, the count was 288; when the optimum concentration of alfalfa extract was added to the medium of Williams the count was 286, but the count was not raised by adding extract of wheat germ or of alfalfa to their medium F. Therefore, they concluded that vitamins are not needed for yeast growth. Williams' medium gave a count of 130 when nothing was added; wheat germ extract raised the yeast count to 188; alfalfa extract raised it to 286, but neither could raise the yeast count when medium F was used. They drew the conclusion that the yeast test has no specificity.

MacDonald and McCollum (1921) carried out an extensive

series of transplantations of pure cultures of yeast in a nutrient medium containing no trace of vitamin B. They showed conclusively that a steady growth through many successive seedings is possible in the absence of the substance for which it had been suggested the growth of yeast could be made a specific test. The only objection which it was possible to raise against these experiments was the possible contamination of cane sugar of the purity of commercial domino sugar. This possibility left doubt in the minds of Funk and Dubin as to the conclusiveness of the data from these experiments. Although samples of sugar purified by repeated precipitations from aqueous solutions by the addition of alcohol were used, the experiments were still criticized on the assumption that such precipitation did not necessarily lead to purification. Fulmer, Nelson and White (1923) have answered this question with finality by employing in place of cane sugar a product, methose, prepared from formaldehyde by the method of Loew. Methose is fermentable and utilizable by yeast. They found that this product could be substituted for cane sugar without impairing the development of yeast in the synthetic medium.

Yeast Synthesizes Vitamin B.—Nelson, Fulmer and Cessna (1921) demonstrated that yeast can synthesize vitamin B when grown in their nutrient media. This was shown by employing such yeast as the sole source of vitamin B in growth experiments with rats kept on diets which had no other source of this substance. MacDonald (1922) fed 5 varieties of yeast grown in a synthetic medium free from vitamin B and confirmed the findings of Nelson, Fulmer and Cessna that yeast can synthesize this substance. Harden and Zilva (1921) found that Saccharomyces ellipsoideus produced more vitamin B in pure cultures than did S. cerevisiae. The latter grew more slowly and imperfectly in the synthetic medium, whereas the former gave a larger yield. They used pigeons for their tests. They fed them a vitamin-free diet until they developed polyneuritis and when such yeast relieved the birds of the retracted neck for at least 3 days they interpreted it as a cure.

Various Extracts on Yeast Growth and Activity.—Von Euler and Pettersson (1921) stated that the rate of galactose and sucrose fermentation by yeast as measured by carbon dioxide production was accelerated by lemon juice and wheat extracts even when the number of cells did not increase appreciably. Some of their experiments may be interpreted to mean that the

formation of zymase is favored by vitamin B. Fränkel and Hager (1921-22) extracted animal organs with 80 per cent alcohol after freeing the tissues from blood and fat. The extracts were tested on yeast and were found to stimulate carbon dioxid production with the exception of bone marrow extract, which had but a feeble effect. Similar studies were made with plant extracts. In general hull and root preparations were weakly stimulating, while leaf mixtures were active in advancing carbon dioxid production by yeast. The most active preparations were from leeks. Roasted coffee was an active stimulant but green coffee was not. Caffein gave negative results. Cholin inhibited as did cholamin. Fuller's earth adsorbed the active principle to a great extent. Kaolin adsorbed it completely, but aluminum hydroxid did not. Active preparations from yeast did not give a Molisch reaction. They considered the stimulating substance to be basic in character.

Eijkman, van Hoogenhuijze and Derks (1922) found that yeast not only takes eventually its anti-neuritic factor from the culture medium but it is not capable of synthesizing the vitamin unless the medium contains at least the products of decomposition of the vitamin produced by heat. They say that the yeast cell is not able to synthesize the anti-neuritic factor but can only regenerate it after it has been denatured by heating. They point out that these results are contrary to those of Nelson and coworkers but state that the vitamin B and the anti-neuritic substance are probably not the same. They state that B. coli communis, even after being cultivated in a medium containing the anti-neuritic substance, remains free from it.

Dietary Properties of Yeast.—Funk pointed out that yeast is a rich source of vitamin B, and this has been repeatedly confirmed by others. Kennedy and Palmer (1922) have found, however, that yeasts from different sources vary considerably in their content of vitamin B, and believe that it should not be used as a standard by which to compare other substances in feeding experiments on mammals.

Nelson, Heller and Fulmer (1923) studied the dietary properties of yeast. They found the protein of yeast, when fed in liberal amounts, to supply all the amino-acids necessary for the nutrition of the rat. Their results from studies designed to show the potency of yeast as a source of vitamin B showed that a diet containing 5 per cent of yeast as the sole source of the vitamin not only induced growth but third generation young were

secured. They concluded from their results that it is unnecessary to postulate the existence of a special vitamin for reproduction (see Evans and Bishop, p. 298). Swoboda (1922) has studied the amino-acid requirements of yeast in relation to the amounts of several of these yielded by hydrolyzed edestin.

Heller (1923) points out that possibly the reason why Eijkman, van Hoogenhuijze and Derks did not succeed in growing yeast on a synthetic medium may be due to the fact that certain synthetic media do not enable yeast to synthesize vitamin B. He raises the question as to whether the character of the synthetic medium influences the amount of vitamin B produced. He cured pigeons with a yeast grown on a synthetic medium. Saccharomyces cerevisiae grown in a synthetic medium is not so rich in vitamin B as when grown in wort. However, it synthesizes the growth-promoting as well as the anti-neuritic substance if the two are distinct.

Levene and Mulford (1923) observed that certain samples of yeast which would be a suitable source of vitamin B for growth of rats did not protect pigeons from polyneuritis when fed polished rice. Five-tenths to two grams a day were given to different birds. They interpreted their results to indicate that there is a growth-promoting substance and another preventing beriberi. Lepeshkin (1924) states that yeast does not grow normally in synthetic solutions containing glucose, asparagin, magnesium sulphate and mono-potassium phosphate when seeded with 1 or 2 cells. When seeded with large quantities of yeast it develops normally. Funk's preparation of vitamin B, when added to the extent of 0.01, 0.001 and 0.0001 per cent, increased growth.

Wildiers' Bios.—Wildiers (1901) observed that when a synthetic medium containing only well defined substances was inoculated with yeast no growth occurred unless the seeding was a large one. He explained the phenomenon by saying that the yeast required some unknown substance for its proliferation which the synthetic medium did not contain but which was present in beer wort, and which yeast cells also contain. He termed this substance "bios" and attempted to study its chemical and physical properties. He concluded that yeast itself does not produce bios, and hence was dependent upon the nutrient medium for its supply. Pringsheim (1906) studied the phenomenon and concluded that the failure of small inoculations to grow in solutions containing only mineral salts and sugar is due to the inability of the yeast to adapt itself rapidly enough to its new

surroundings. When a larger seeding was used some cells budded at the expense of nutrients derived from the dissolution of other cells. He stated that in a few generations these became adapted to the assimilation of the unaccustomed medium and were then able to grow. After repeated inoculations in the synthetic medium single cells of wine yeast were observed to develop into colonies. He regarded Wildiers' bios simply as a suitable source of nitrogen for use of the yeast while adapting itself to the synthetic medium.

Wright (1921) described experiments consistent with this explanation. He found that when small amounts of yeast extract were added to the synthetic medium the yeast grew at the expense of the nutrient principles supplied by the extract until the concentration of cells reached a certain magnitude, after which budding could go on through the utilization of the nitrogen of ammonium salts.

Question of Identity of Vitamin B and Bios.—Lecoq (1920) asserted that the bios of Wildiers was not identical with vitamin B, but was probably the same as the auximones described by Bottomley (1914) which stimulate plant growth. Funk and Dubin (1921) separated from an extract containing vitamin B a substance which they called vitamin D, which they believe to be necessary for the growth of certain microorganisms. Their vitamin D will be understood as the equivalent of bios. Funk and Dubin questioned whether vitamin B and the anti-neuritic substance are identical and also whether D was a distinct entity essential for yeast and certain bacteria. They stated that pigeons need only B, whereas rats require both B and D. These investigators also reported that autolyzed yeast shaken with fuller's earth retains most of the substance responsible for the acceleration of the growth of yeast.

MacDonald and McCollum (1921) described experiments in which yeast was grown in a purified nutrient solution of mineral salts and cane sugar of high purity, sufficient to furnish from 2 to 5 gm. of dry yeast cells. Successive seedings from a few cells into the synthetic medium were carried so far that a culture was secured which contained less than one-five trillionth of the bios in one loop of yeast cells used in the first seeding. Assuming the freedom of the cane sugar employed from bios or from the anti-neuritic factor, it appeared that yeast may grow without either of these substances or it must be able to synthesize its own supply.

Ide (1921) called attention to the fact that there are two kinds of proliferation of yeasts, one very slow without, and one fast with bios. Without bios he observed slow proliferation and says that after several weeks weighable quantities of yeast were obtained. This had been observed by many others. Ide suggested that the proliferation of yeast in the experiments of MacDonald and McCollum was due to impure cultures. It was stated by MacDonald and McCollum, however, that "The successive seeding was continued to the fifteenth seeding with no apparent change in the rate of growth or diminution in the fermentative activity of the yeast. Every precaution was taken to insure pure seedings, a microscopic examination of a sample from each flask being made before seeding the next flask because of the known influence of other fungi."

Fulmer and Nelson (1922) found that yeast grew so satisfactorily in their medium F, that the addition of vitamin B did not improve it. The addition of water extracts of alfalfa did stimulate the growth of yeast in medium F. This extract evidently contains the bios of Wildiers, along with vitamin B and other materials. They say that 95 per cent alcohol extracts vitamin B but not bios, and that a 95 per cent alcoholic extract of alfalfa does not improve medium F for the support of growth of yeast. This confirms their previous work.

Eddy, Heft and Stevenson (1922) take issue with Fulmer and Nelson on certain points. They state that Fulmer and Nelson believe that "water extracts both bios and vitamin B, but alcohol extracts only vitamin B." Eddy and co-workers apparently have had stimulation of yeast with their 95 per cent alcoholic extract, although the stimulation was not so great as with the water extract. They say "Our own results, including those accumulated since the publication of our paper, have only served to increase our distrust of the test as an accurate measure of vitamin B."

Wright (1922) used very small amounts of lemon juice with a mineral nutrient solution for the growth of yeast cells. He stated that his observations point to the fact that bios does not enable yeast to assimilate ammonium sulphate nitrogen simply by its presence or by being consumed at the same time, but that the yeast grows slowly at the expense of the bios until it reaches a certain concentration, and after that it is able to use the ammonium sulphate.

Freedman and Funk (1922) stated that they obtained from purified casein, commercial gelatin, yeast protein and edestin by

hydrolysis, certain substances which showed marked growth stimulating activity on hemolytic streptococci. Hydrolosates of purified egg albumin and hordein showed traces, whereas hydrolosates of other proteins examined were devoid of these activating substances. The properties of the latter were such that they believed them to be related to, if not identical with, the vitamin B obtained from brewer's yeast. The similarity of this substance to vitamin D was particularly marked. They also found that there is present in beef and beef heart infusions, and in peptone and autolyzed yeast, a substance which shows comparable growth stimulation on hemolytic streptococci and yeast cells. This they speak of as the vitamin D.

Funk and Paton (1922) report that pigeons were kept over half a year on polished rice plus a highly purified vitamin B preparation. This they interpreted to indicate that adult pigeons need only vitamin B and can dispense with all others. The fractions used were all purified fractions of vitamin B practically devoid of vitamins A and D. They believe that the pigeon is more suited as a test organism for vitamin B preparations than the rat because of the more complicated requirements for vitamins in the latter. They claim that rats fail when vitamin B devoid of vitamin D (bios) is provided. Parallel experiments were carried out on the pigeon and the rat, and the rat responded but the response was of a smaller magnitude than when vitamin B was given in yeast. They believe that the test period of the rat can be no less than 14 days, and if then the change is not significant the fraction is to be regarded as devoid of or deficient in vitamin B. They interpret their experiments with pigeons and rats to mean that the growth of yeast or certain other fungi takes vitamin D out of the nutrient medium but leaves vitamin B behind. They state that yeast cells retain the vitamin B very tenaciously after they have taken it out of solution. These investigators express the view that the rat requires both B and D, whereas the pigeon requires only B. It should be emphasized that their term vitamin D is equivalent to bios.

Kinugasa and Hattori (1922) support the view with numerous experiments that the anti-neuritic substance is not identical with that which stimulates the growth of yeast. They believe the yeast growth-promoting substance is a component of the ordinary preparation called vitamin B, and is distinct from the latter. This view is concurred in by Heaton (1922) and Hoet (1922).

Funk, Harrow and Paton (1923) extracted yeast with 70 per

cent alcohol, and found the residue inactive for vitamin B. Acetone was the best solvent for giving small amounts of nitrogenous impurities in the extract but it also left behind an active residue. When the nitrogen content was high there was a high vitamin content. They state that vitamins B and D tend to run parallel in their preparations. Co-ferment showed no relation to either B or D. When they used rice polishings 60 per cent was better than 70 per cent alcohol. Suzuki and Suzuki (1923) state that from oryzanin (a vitamin B preparation) by the use of aluminum cream and other precipitating agents they separated vitamin D (bios) which promotes bacterial growth. They state that B acts both on the higher animals (anti-neuritic substance) and on lower organisms (bacterial growth factor), while D acts only on the latter.

MacDonald (1923) stated that extracts of yeast grown in a distilled water solution of pure cane sugar and nutrient salts served to stimulate the growth of seedings in such a medium in a manner comparable in its effectiveness with that of extracts of commercial yeast. Apparently yeast synthesizes a substance which stimulates the growth of the culture. This is the bios of Wildiers. It does not act as a vitamin, for an animal cannot grow without the necessary vitamins, whereas yeast can grow without bios. She further states that small seedings of yeast cells in the new environment of a fresh inoculation in a synthetic medium grow very slowly but proliferate much more rapidly when the special nutrient principle is supplied. Bios apparently does not act like a vitamin, since an animal can not survive unless the vitamin is present but yeast can dispense with bios, although it grows much more rapidly in its presence. It can synthesize it but does so slowly.

Growth of Yeast Using Synthetic Sugar, Methose.—Funk and Freedman (1923) claim that cane sugar carries vitamin D (bios) and that without it the strain which they used could not synthesize vitamin B. According to this view yeast cannot synthesize vitamin B in the absence of D, for, they say, in the absence of bios, yeast cannot grow. In this condition it should be recalled that Fulmer, Nelson and White (1923) have successfully grown yeast in a synthetic medium comparable to that used by the other investigators mentioned, but in which methose, a synthetic carbohydrate prepared from formaldehyde, replaced the cane sugar. This lends strong support to the correctness of MacDonald's view.

Williman and Olson (1923) report that vitamin B is readily soluble in 95 per cent alcohol, whereas bios is not. They list 60 nitrogen compounds which have been investigated, including 19 which they tested themselves. None of these were found to be bios.

Fractionation and Isolation of Bios.—Devloe (1906), Ide (1907) and Frankel and Schwartz (1920) describe the fractionation of various extracts with a view to the isolation of bios. They appear to have assumed that bios is a single substance. Miller (1924) has presented evidence indicating the multiple nature of bios. With this view Fulmer, Duecker and Nelson (1924) are in agreement. They have separated a growth-stimulating preparation into 2 fractions, neither of which alone produces maximum stimulation, but do so when added together.

Eddy, Kerr and Williams (1924) have isolated from yeast a crystalline substance melting sharply at 223° C. They believe this to be bios. The recrystallized product retains its melting point and shows no loss of activity. The yield of the product was about 0.03 per cent of the dry yeast. It contains 43.29 per cent of carbon and 8.31 per cent of hydrogen. The nitrogen content has not been accurately determined but is about 25 per cent. The substance gives no ninhydrin or biuret reaction. It is easily soluble in cold water, in acid and alkaline solution, as well as in dilute ethyl alcohol. In cold 95 per cent alcohol it is sparingly soluble but passes readily into solution when the alcohol is heated. It is only slightly soluble in 100 per cent acetone and very slight dilution with water brings about its solution. The essential feature of the method of isolation consists of a preliminary purification of autolyzed yeast with fuller's earth which removes substances other than bios. Then the use of ferric hydroxid in colloidal solution under specific conditions removes the bios which can later be dissolved and the bios freed from contaminating ions.

Levene and van der Hoeven (1924) call attention to the fact that many years ago Mandel and Dunham prepared from a commercial preparation zymase, and adenin hexosid. Levene has recently prepared a considerable quantity of this from yeast. Its composition is C 44.41, H 5.09, N 23.57 per cent. Its melting point is 214°. Levene points out that the analytical data, except for the hydrogen, remind one of those given by Eddy and coworkers. The adenin hexosid was tested by Levene as a source of vitamin B for yeast growth but was not active. Levene

states that possibly the similarity in the elementary composition of bios and of adenin hexosid is accidental.

Deas (1924) using malt combings which consist largely of sprouts of malt, prepared extracts from which two substances called bios I and bios II were isolated. Bios I may be precipitated with barium hydroxid and is not adsorbed on charcoal, nor is it precipitated by lead acetate except in the presence of ammonia. It is not removed by shaking with yeast. Bios II is absorbed by charcoal and is removed from solution by shaking with yeast, and is soluble in acetone. Excess of either of these two substances, especially bios II, is detrimental to the growth of yeast. Rats fed a diet deficient only in vitamin B were given 1 gm. daily of the rootlets in the form of a powder. Others were given bios I, others bios II, and others bios I and II in the same doses. Some rats were given an infusion of the rootlets. The results showed that neither the rootlets nor the infusion contained vitamin B. Bios I and bios II, or both in combination so balanced as to promote abundant growth in yeast, were also inefficient in promoting the growth of rats. The substances do not cure beri-beri in pigeons. He draws the conclusion that Wildiers' bios and Funk's vitamin D are not identical with the rat growth-promoting vitamin B.

Unidentified Nutrients Essential for Microorganisms.—Pacini and Russell (1918), working with rats, apparently showed that the typhoid bacillus produces vitamin B. Their experimental work is not convincing. Damon (1921) demonstrated that B. paratyphosis beta, B. coli, and B. subtilus do not produce vitamin B synthetically. Leichtentritt (1921) stated that malt, lemon and carrot extracts exert a stimulating effect on the growth of bacteria, especially staphylococci and diphtheria bacilli. This effect is not exerted by blood or ascitic fluid. György (1921) reported that "accessory substance" from milk and vegetables has the property of increasing cell respiration in isolated cells.

Thjötta and Avery (1921) found that the growth of B. influenzae depends on two distinct and separate substances which are present in the blood. These occur in plant as well as in animal tissues, and sterile raw potato will serve as a substitute for blood in the cultivation of this bacillus. Broadhurst (1921) reports that approximately 20 species of bacteria on agar cultures from 2 to 24 weeks old were revived by a sterile vitamin B extract of navy bean. Walker (1922) found that some of the more highly parasitic bacteria, *e.g.*, the gonococcus, refuse to grow or only

grow with difficulty on the ordinary culture media. Alcohol-ether extract of egg yolk or of fluids in which bacteria have grown and autolyzed for some time makes possible the growth of such bacteria. It is suggested that while most bacteria can produce for themselves any vitamins which they require certain parasitic forms have lost this power. Kuroya and Hosoya (1923) discuss the synthesis of vitamin B by coli bacillus grown on synthetic medium. They state that if B. coli is grown on their synthetic medium, and is then given by mouth to pigeons suffering from polyneuritis the diseased pigeons are cured promptly.

Damon (1923) gives a review of the literature on the presence of growth-promoting substances in bacteria. He objects to the term vitamin as applied to any growth-stimulating substance of bacterial origin until such substance has been subjected to the biological test with a mammal. He found that 5 per cent of a spore-forming organism, B. adhoerens, and 5 per cent of a mucoid organism, Friedländer's bacillus, were found not to influence growth in young rats. Five per cent of Pfeiffer's bacillus, another mucoid organism, added to a diet deficient in vitamin B, maintained animals at a constant weight or induced rapid growth. Five per cent of B. timothy, an acid fast organism, had the same effect as Pfeiffer's bacillus. Ten per cent of B. timothy induced rapid and continuous growth in rats.

Scheunert and Schieblich (1923) fed pigeons a vitamin-free diet until there was a marked decrease in weight and body temperature. They were then fed B. vulgatus which had been grown on a vitamin-free bouillon. A marked increase in body weight and rise in temperature followed. They concluded that the B. vulgatus can synthesize the vitamin B from vitamin-free substances. They believe the vitamin is in the cell body and not in the nutrient medium as a product of metabolism. Hosoya and Kuroya (1923) studied the effect on bacterial growth of a vitamin B preparation prepared as a silver compound from the histidin fraction of the phosphotungstic acid precipitate of an alcoholic extract of bran. They describe a protein-free nutrient solution in which a considerable number of bacteria fail to grow appreciably, but are stimulated to growth by the addition to the medium of very small amounts of the vitamin preparation. They state that they are the first to obtain a protein-free synthetic medium suitable for the growth of meningococci. Shiba (1923) reported that the presence of vitamins A, B and C greatly favors the growth of B. typhosis, staphylococcus and B. anthrax. Wyon

(1923) does not believe the vitamin B is essential to bacteria but believes that the amino-acid content of the media is more important.

Drew (1923) found that substances necessary for continuous growth which are present in extracts of embryonic tissues are able to pass a Berkefeld filter. Adult tissues and tumors appear to contain these substances in lower concentration. Carrel (1924), in his studies of *in vitro* growth of tissues, emphasizes the importance of unidentified substances in the nutrient medium. As far back as 1867 Claude Bernard defined internal secretions as being nutrient secretions preparing immediate principles for cell nutrition, such as glycogen, albumin and fibrin. Carrel says that leucocytes, according to their condition, set free substances of varied nature. Trephones are secreted by normal cells or diffuse from dead cells before the onset of disintegration.

Damon (1924) states that certain members of the acid fast groups of bacteria produce in themselves substances which promote growth in rats. A growth-promoting substance analogous to vitamin B was found to be present in the following acid-fast bacteria: B. smegmatis; B. timothy; B. moelleri. These were fed at 7½ per cent of the food mixture. Slanetz (1924) states that the following organisms produce neither A nor B as determined by feeding experiments with rats: Azotobacter chroöcoccum; B. lactis acidi; B. mycoides; B. prodigiosus; B. radicicola; B. subtilus and Micrococcus agilis.

Observations on Vitamin Requirements of Plants.—Only a few studies have been made to determine the influence of unidentified nutrient principles on plant growth. Livingston (1907) discovered that organic material may be of direct importance when experimenting on the effect on plant growth of extracts of green and stable manures. He added these substances to a slightly modified Knop's solution and found that "by far the most important factor in the effectiveness was the organic matter," which he found to be of direct value to the plant. Bottomley (1914) found that growth of green plants (lemma minor) in water culture was better with the addition to the inorganic nutrients of certain organic substances. He found these to be absolutely essential if the plant was to grow healthily and normally for any length of time. He called these substances auximones. They apparently function similarly to vitamins. He found that they were produced very largely during the bacteriological decomposition of peat by aerobic organisms, and that they

were water-soluble, being extracted from peat by water. Mockeridge (1920) found that ordinary organic manures which are generally applied to soils have been shown to contain water-soluble substances which promote growth in plants (auximones). See confirms the work of Livingston and Bottomley.

It will be clear from what has been said that the nutritive requirements of the microorganisms have no similarity to those of mammals. It is possible that a series of unidentified substances will be found to be factors in determining whether certain species of bacteria or fungi can thrive in any medium.

CHAPTER XV

THE DIETARY DEFICIENCY DISEASES

Xerophthalmia (*Keratomalacia*)

Investigations on Lipin-free Foods.—Stepp (1909) in the laboratory of Hofmeister carried out the first classic experiment which showed the indispensability of some substance contained in lipoid extracts of certain natural foods. He believed the fat-like bodies, the lipins, under which term is included lecithin, kephalin, cholesterol, cerebron, etc., to be primary and indispensable cell components. Most food-stuffs of animal or vegetable origin contain these lipins, hence they occur regularly in our diet. Stepp sought to discover what would happen if they were eliminated from an otherwise adequate food mixture.

Lecithin contains combined in its molecule glycerin, fatty acid, phosphoric acid, and an organic base called cholin. Cephalin closely resembles lecithin, but yields on hydrolysis a different organic base, amino ethyl alcohol. Cholesterol, chemically an alcohol, has certain physical resemblances to the fats. Cerebrosides are compounds containing in their molecules a fatty acid, a sugar (galactose) and an organic base (sphingosin). All of these are soluble in alcohol, but they differ to some extent in their solubility in the other solvents named. All of these substances are abundant in brain and nerve tissue.

Stepp prepared a bread from milk and wheat flour, and another from milk and "protomol," a protein-rich preparation from rice. These he extracted thoroughly with alcohol and with ether. Mice were fed either the extracted or the unextracted breads. Those fed the extracted food died within 30 days, whereas those fed the untreated bread remained in good health during the 6 or 8 weeks they were under observation. Stepp interpreted these results to mean that certain lipins are essential in the diet. He next sought to discover which of the individual lipins was indispensable for the maintenance of health.

The method employed was to restrict mice to the lipin-free diet plus one or more of the individual lipins which can be prepared

in a state of purity. He tested lecithin, cholesterol, cephalin and cerebrosids, but was unsuccessful in making the extracted diet complete by the addition of one or more of these purified substances. He made an extract of egg yolk by shaking with cold alcohol, and divided the solution into two portions. One of these was heated with 95 per cent alcohol for 2 days in a water bath, while the other was heated only long enough to evaporate the alcohol. Each of these preparations was added to the extracted bread and the two mixtures were fed to mice. Those fed the heated preparation died within 30 days, whereas those receiving the unheated preparation remained in health. The substances which dissolved from the bread when it was treated with alcohol and with ether were changed by prolonged heating, so that they no longer rendered the extracted breads complete for the nutrition of mice.

Birds Produce Synthetically Fats and Complex Lipins.—It was shown by Fingerling (1912) and independently by McCollum, Drescher and Halpin (1912) that birds may be satisfactorily nourished on diets very poor in lipins. The latter fed three half-grown hens exclusively on a mixture of polished rice and skim milk powder, the latter having been extracted with ether to render it essentially free from fats. On this diet the hens increased their body weights about 33 per cent, and laid 57 eggs. These eggs collectively contained 82.95 gm. of lecithin and cephalin. This is several times the amount of these substances contained in the entire bodies of the hens. Both neutral fats and complex lipins must have been produced synthetically by the birds.

Fingerling fed ducks a diet consisting of 300 gm. of cooked potatoes, 80 gm. of cooked blood albumin, 300 gm. of boiled starch, 10 gm. of calcium phosphate and 10 gm. of calcium carbonate per bird per day. The ducks were full grown. One duck was estimated to have deposited in her eggs 302.3 gm. of lecithin, another 246.0 gm. and another 212.7 gm. The birds remained in health during the egg-laying period. There was no doubt that the ducks had synthesized large amounts of complex lipins.

Do Nutritive Requirements of Pigeons Differ from Mammals?—Stepp (1916) inquired whether pigeons differ in their nutritive requirements from mammals. He fed 3 pigeons exclusively on dog biscuit which was extracted with hot alcohol. All were dead within 43 days of polyneuritis. A pigeon on the same food was unable to stand on the thirty-eighth day because

of polyneuritis. It was then given doses of a commercial "vitamin" preparation (oryphan) plus an alcoholic extract of egg yolk to furnish the lipins which he believed to be indispensable for mammals. The bird rapidly recovered and remained in good condition until the ninety-second day of the experiment. It was then fed unextracted dog biscuit until the ninety-eighth day. The diet was then changed to extracted dog biscuit supplemented with oryphan but without the lipin extract. The body weight at this time was 271 gm. It had been on experimental diets for 117 days. On this lipin-free diet it steadily, but slowly, lost weight so that by the one hundred and ninety-third day it weighed but 217 gm. It still ate well, but its flight was unsteady. Unextracted dog biscuit was fed for 2 days. The pigeon was then given the extracted dog biscuit without the addition of either lipins or "vitamins." The result was a rapid loss in weight, and the development of polyneuritis. Death occurred on the two hundred and thirty-third day.

From these observations Stepp drew the unwarranted conclusion that the bird does not require lipins which are indispensable for mice. The loss of weight of his pigeon shows that its nutrition during this part of the experiment was not satisfactory. These results do not warrant the conclusion that there is a difference in the nutritive requirements of birds and mammals.

In Stepp's investigations the element of growth was not included. He studied the nutritive requirements for maintenance of adult mice and pigeons. His results are, nevertheless, of great importance. Stepp, in his early studies, interpreted the results to indicate that only certain lipins were necessary to supplement successfully a diet of purified food substances. When Funk demonstrated the necessity of a protective vitamin for the prevention of beri-beri, Stepp extended his studies to include "vitamins" in addition to lipins. In November, 1915, he presented data showing the necessity of "vitamins" as well as certain lipins in the diet for maintenance. McCollum and Davis simultaneously demonstrated the necessity for growth or maintenance in rats of a fat-soluble and a water-soluble substance.

Earlier Views on Fat in Diet.—McArthur and Luckett (1915) misjudged the peculiar nutritive value of butter fat as a source of an unidentified dietary essential. They added butter fat to a diet free from substances soluble in ether and alcohol, and observed that mice died as early as they did on the lipin-free diet. The ration was made adequate by the addition of an alco-

holic extract of egg yolk or calf brain. These investigators, like Stepp, drew the conclusion that butter does not contain a substance indispensable for the nutrition of the mouse. The explanation of their results was finally brought out by the studies of McCollum and Davis (1915). When butter fat or an ether extract of egg yolk was added to a diet of purified food-stuffs, only one of the unidentified dietary essentials (vitamin A) was introduced. When an alcoholic extract of egg yolk was added, both vitamins A and B were added. Since the addition of either the factors A or B alone does not make a diet of purified food-stuffs complete, the animals fed one addition died, and there was no evidence to show that the butter fat had any special properties. It was difficult to discover that in the one procedure one essential substance, and in the other two, were being added, and that the presence of the two was the determining factor in the outcome of the experiment.

Osborne and Mendel (1920) made a study of diets poor in fats. Rats were fed a dry mixture of starch paste 72, meat residue thoroughly extracted 23, and salt mixture 5 per cent. This was fed *ad libitum*. The animals also received daily 0.4 gm. of brewer's yeast, 0.2 gm. of alfalfa leaves, as a source of the vitamins A and B, and also 0.2 gm. of cane sugar. The control animals were fed a similar diet except that the meat residue was extracted 5 times with ether containing a little ethyl alcohol. These rats grew as rapidly as on normal diets. The results warranted the conclusion that if true fats are essential for nutrition during growth the minimum necessary must be exceedingly small.

Drummond and Coward (1921) found that young rats could grow from weaning to maturity on diets deprived as far as possible of neutral fats. The animals developed normally. They say "It would appear that neutral fats, from a purely physiological standpoint, are dispensable constituents of a diet, provided a sufficiency of the vitamin frequently found in association with natural fats is contained in the other food-stuffs. The real value of fats as convenient sources of energy is obvious."

It is obvious, from the data presented above, that the peculiar value of certain fats for the promotion of growth or of health, does not lie in their fats or complex lipins, but must be due to the presence in them of some substance of an unknown nature. This substance we call vitamin A.

Xerophthalmia in Man.—When animals are restricted to a

diet lacking in vitamin A, there develops after a few weeks a pathological condition of the eyes. This is so characteristic that it must be regarded as one of the manifestations of a specific syndrome. Livingstone (1857) in describing the hardships endured in his explorations in Africa, relates that his party, while suffering from exposure to the weather, lived for a time on sugarless coffee, manioc roots and meal, and states "the eyes became affected (as in the case of animals fed on experiment on pure gluten or starch)." We have been unable to discover a description of the original experiments with which Livingstone was familiar. It is remarkable that at this early date he was familiar with the xerophthalmia which results from faulty diet. Spicer (1892) states that after measles or whooping cough with bronchitis when there had been serious exhaustion, the cornea not infrequently became damaged by large perforating ulcers. He states that after serious malnutrition the cornea might slough spontaneously as was not uncommonly seen among nurslings where the mothers practiced long religious fasts. In this country it was rare except among the hand-reared children who had insufficient nitrogenous diet. The affection generally attacked both eyes of children from 4 to 9 months old; it began with a dryness of the conjunctiva with patches of froth on its surface and with night blindness. Soon the whole cornea became opaque and perforated. The termination was often fatal. In the treatment which he applied the principal feature was to increase the nitrogenous portion of the diet with meat juice or raw chopped meat in addition to supplying meat and cod liver oil.

Mori (1904) described fourteen hundred cases of xerophthalmia among children between the ages of 2 and 5 years in Japan in 1905-1907. They exhibited keratomalacia as well as xerosis of the conjunctiva. This syndrome is known in Japan as hikan. Mori states that the disease does not occur among fisher folk, but among people whose diet is largely of vegetable origin. The children suffered from diarrhea in the summer time in addition to the eye lesion. The diet consisted of "rice, barley, cereals, beans and other vegetables." The nature of the other vegetables and their relative amounts were not stated. Prompt relief of the eye symptoms was observed when cod liver oil was administered. Chicken livers and eel fat were also found to be effective as a remedy. The successful treatment of xerophthalmia in these children by the administration of cod liver oil or by the feeding

FIG. 9.—Illustrates a baby observed and treated by Dr. C. E. Bloch, of Copenhagen. The child was suffering from severe xerophthalmia due to being confined too largely to a diet of separator skim milk supplemented with cereals. The right eye is filled with leucocytes and presents a yellow appearance. The left is swollen shut. The right eye was affected with a corneal ulcer. The sight of the right eye was lost.

FIG. 10.—Illustrates a baby from the practice of Dr. Bloch, which had suffered from an attack of ophthalmia of dietary origin, and was cured by administration of fat-soluble A as butter and cod-liver oil. The disease had, however, progressed so far that the sight of the left eye was destroyed. The right eye was somewhat damaged.

of a glandular organ, such as liver, affords very strong evidence that the ophthalmia was the same condition as has been produced experimentally in animals.

Experimental Xerophthalmia in Animals.—Falta and Noeggerath (1906) before the vitamin hypothesis was formulated described a conjunctivitis with crust formation beginning about the fourth week and lasting until death in rats which were fed a diet which from their description must have been very deficient in vitamin A. Knapp (1909) restricted rats to a diet of purified food substances and described a severe conjunctivitis which developed as the nutrition of his animals failed. In other experiments lecithin, cholesterol and nucleic acid were added to the basal diet, and although the animals suffered from malnutrition and died, the characteristic eye symptoms did not appear. This is probably to be explained by the presence of vitamin A as an impurity in the lipins which he employed.

Freise, Goldschmidt and Frank (1913) observed the same eye disorder in experiments with rats which they restricted to a diet similar to that used by Knapp. They stated that in about 3 weeks from the beginning of the experiment the eyelashes of the animals began to fall out. There was retraction of the eyeballs from spasm of the extrinsic muscles. In 5 to 6 weeks the sclerotic coat became dry, the cornea clouded and finally ulcerated, but without marked inflammation. The fur became rough. They succumbed to malnutrition. Histological examination of the sclerotic coat revealed keratomalacia as it is seen in man. In cases where the ulceration of the sclera of one eye was well developed, and was beginning to show in the other, the process could be entirely arrested by the administration, suggested by the work of Hopkins, of 2 c.c. of skim milk per day. These workers believed that the condition developed only in young growing animals, and that it was closely related to the phenomena of growth. They attributed the condition to lack of "accessory" substance in the diet.

Osborne and Mendel (1913) called attention to the prevalence of inflammation of the eyes of rats restricted to a diet of purified proteins, "protein-free milk," lard and starch. They referred to this as a "type of nutritive deficiency exemplified in the form of an infectious eye disease prevalent in animals inappropriately fed," and stated that the condition was speedily alleviated by the introduction of butter-fat into the diet.

Xerophthalmia in Danish Children.—Bloch (1917) described 50 cases of xerophthalmia in children in the vicinity of Copenhagen during the years 1912-16. Most cases were infants under one year of age. They were suffering from severe malnutrition. The skin was dry, shriveled and scaly. Their diet consisted of separator skim milk, practically free from fat. The milk had been pasteurized and cooked again in the home before it was fed. Oatmeal gruel and barley soup constituted the other important ingredients of the diet. These infants were doubtless suffering from several faulty dietary factors. Such a diet would contain relatively little vitamin A, the amount depending on the nature of the feed of the cows; the thoroughness of skimming; the amount of skim milk which was consumed, and the extent to which the heat treatment destroyed it. If the consumption of cereal was large, the amount of milk taken would be too small to insure satisfactory inorganic content in the diet. The diet which Bloch described would be practically free from anti-scorbutic substance. The infants were probably in a borderline condition of scurvy and beri-beri along with very pronounced ophthalmia. *In the past we have failed to appreciate the seriousness of the condition of malnutrition which falls but little short of a deficiency disease.* The effects on vitality may be very pronounced in cases where a faulty diet is adhered to until clinically observable symptoms are about ready to appear, but while no clear cut lesions are detectable.

Bloch referred the malnutrition in these infants to lack of fat in the diet, since the eye trouble could be relieved by the administration of cod liver oil, whole milk or cream mixtures. In the light of our present knowledge there is no reasonable doubt that vegetable fats such as olive oil, cotton-seed oil, soy bean oil, coconut oil, etc., would not have been of any therapeutic value. The prominence of skim milk suggested to Bloch the paucity of fat in the diet, and he fortunately chose two effective fats, cod liver oil and butter fat for administration to his infants. Bloch (1919) divided 32 healthy children of ages 1 to 4 years in an institution in Copenhagen into two groups for nursing and feeding. In group 1 during May and June, 8 cases of xerosis developed, while those in group 2 remained free from the disease. The diets were similar except the breakfasts. Those in group 1 were given oatmeal porridge and rusks, whereas those in group

FIG. 11.—Illustrates the appearance of the exterior of the eyes as ophthalmia of dietary origin develops. On the left is shown the normal eye of the rat. In the center is shown the puffiness around the eyes which denotes that ophthalmia is about to appear. On the right the extreme edema of the eyelids is shown. When the latter condition is reached the cornea usually ulcerates and a bloody exudate seals the lids shut. The eyes become filled with leucocytes. The introduction of a fat containing vitamin A leads, in the earlier stages, to prompt recovery from this condition.

FIG. 12.—The rations of these two rats from weaning time were exactly alike except in the character of the fat which they contained. The one on the left was given 5 per cent of sunflower seed oil. The one on the right was given 1.5 per cent of butter fat. Butter fat, egg yolk fats and the leaves of plants contain a dietary essential, the chemical nature of which is still unknown, which is necessary for growth or the maintenance of health. This substance is known as vitamin A, and is not found in any fats or oils of vegetable origin. A lack of this substance in the diet causes the development of a peculiar eye disease known as xerophthalmia. The animals were the same age when photographed.

2 were given beer soup and some whole milk. Apart from the whole milk given to the children in group 2 the only fat supplied was margarine from vegetable fats. Thus, unwittingly a human experiment was carried out *en masse* corresponding exactly to feeding experiments on animals. The fat in the whole milk in group 2 protected the children against xerophthalmia. The ophthalmia was cured with cod liver oil. Bloch (1924) reports a clinical experience with xerosis in Danish children in which an analysis of 100 cases observed between 1909 and 1920 is given.

Vitamin A Starvation and Xerophthalmia in Rats.—McCollum and Simmonds had repeatedly observed the development of this peculiar form of ophthalmia in animals subjected to their faulty diets. They collected numerous observations, all of which pointed to the belief that the eye disease in question is non-infectious, that it could more satisfactorily be explained on the basis of specific starvation for vitamin A than on any other. Many groups of rats in various degrees of enfeeblement or arrested development resulting from restriction to faulty diets of various kinds, failed to develop the disease, when others in adjacent cages were suffering from it. It occurred only in those animals whose diets were deficient in vitamin A. We (1917) correlated these observations on rats with those reported by Bloch and by Mori, and came to the conclusion that the xerophthalmia or keratomalacia produced experimentally in animals is the analogue of the condition which these authors have reported in man. On this evidence they formulated the view that this type of xerophthalmia is a manifestation of a deficiency disease due specifically to lack of vitamin A.

Requirements of Adult Rats for Vitamin A.—Drummond (1919) stated that adult rats need little if any of the vitamin A for the maintenance of health. Stepp's observations indicate the reverse. McCollum and Simmonds (1917) found, when the sole source of vitamin A was 33 per cent of millet seed in the food mixture, adult rats developed after 60 days severe xerophthalmia, although this seed contains demonstrable amounts of vitamin A. It is possible that adults require less of this factor for maintenance than young animals for growth, but this is not proven. An animal on a diet satisfactory in all respects other than in its content of vitamin A will not show signs of a lack of this substance for a much longer time than would be necessary to make the shortage apparent if a second factor in the diet were below the optimum in quality and quantity. The extent to which this

second factor deviates from the optimum determines whether it would promptly or slowly show the effects of faulty nutrition. If at the same time there should exist a deficiency in a third factor, the effects of lack of vitamin A would become increasingly apparent. In the interpretation of quality in the diet it is essential to realize that there is an inter-relation of factors. In short experiments it is easily possible to overlook deficiencies which are of small magnitude, which, nevertheless, manifest themselves later in the life history of the individual.

Vitamin A Requirements of Infants.—Hess and Unger (1920) hold the view that in human nutrition there is not likely to be a deficiency of vitamin A sufficiently pronounced to be a factor of importance except under exceptional circumstances—as in times of war. They base their conclusion on an experiment conducted with 5 infants who were fed dried skimmed milk (krystalack) 150 gm.; sucrose 30 gm.; cotton-seed oil 30 c.c.; autolyzed yeast 15-30 c.c.; orange juice and cereal. The autolyzed yeast was to insure a liberal supply of the anti-neuritic substance, vitamin B. The orange juice was to supply vitamin C, and the olive oil to furnish fat. The diet was assumed to be almost free from vitamin A, but entirely adequate otherwise. The babies were from 4 to 9 months old. They were confined to this diet for periods varying from 5 to 9 months. Rickets were not observed in any of the babies. In fact, they grew in a fairly normal manner. From this Hess and Unger were led to doubt whether more than traces of vitamin A are necessary in the nutrition of the human infant when other factors in the diet are satisfactory. The assumption that the diet employed was nearly devoid of vitamin A is erroneous. Ross (1921) described 4 cases of nutritional keratomalacia in infants and gives a review of the literature pertaining to the disease. The children observed by him had been fed almost exclusively on condensed milk.

Eye Affections in English Schools Referable to Faulty Diet.—Cramer (1924) describes epidemics of eye affections which occurred in England during the last 40 years in various industrial schools. The last recorded outbreak was in 1911 and was investigated by McGowan and McNeal (1913). The condition of the children was diagnosed as "distorted pneumonia" which varied from a fulminating rapidly fatal type to an abortive or latent type. Special mention was made of the prevalence of a chronic granular conjunctivitis. McGowan and McNeal clearly recognized the non-contagious nature of the epidemic but were unable

to explain the nature of the disease or recommend rational treatment.

Butter Consumption and Xerophthalmia in Denmark.—Widmark (1924) states that the consumption of milk fat in Denmark before the war and to the end of 1916 steadily declined and reached very low levels in the last year. At the same time the number of cases of xerophthalmia reached their maximum. Then came a complete change. In 1917 and 1918, and partly also during 1919, the exportation of butter was forbidden. More milk fat was consumed in the country than in the years before the war. Xerophthalmia disappeared almost completely but reappeared in 1920 when the export of butter again began.

Deficiency Diseases Rarely Due to Single Dietary Fault.—It is doubtful whether a deficiency disease ever occurs uncomplicated in man or animals. When the list of foods available is greatly restricted and the diet unsatisfactory to a pronounced degree the pathological condition produced is a complicated one. The specific deficiency diseases, with the possible exception of scurvy, can be produced as uncomplicated syndromes only by carefully planned experimental diets. This is evident when one understands the nature of the deficiencies of polished rice, muscle meats and refined cereal products, the prominent articles in the diet of those peoples who suffer most from beri-beri. Such diets are deficient in mineral elements to a degree that would in time lower the vitality. Their content of vitamin A is so low as to produce a borderline, if not an actual condition of keratomalacia. The defect which produces the most prompt and most visible pathological picture is that resulting from the lack of the antineuritic substance. Beri-beri appears and overshadows in the eyes of observers all other features of general malnutrition.

In the reported cases of xerophthalmia in man, the inference seems justified that, although many of the infants were doubtless suffering from latent scurvy and under-feeding, lack of sufficient vitamin A was a prominent factor in their malnutrition.

Vitamin A and Night Blindness.—Another widespread condition which seems related to a deficiency of vitamin A in the diet of man is hemeralopia, or inability to see by subdued light. This is common in several parts of the world, and has been frequently referred to faulty nutrition. Thus Krienes (1896) attributed the disease to malnutrition. DeGouvea (1883) gave a résumé of the literature of the subject, and described many cases of hemeralopia among the negro slaves working the coffee plan-

tations of San Paulo, Brazil. They were restricted to a diet of beans, pork fat and maize meal. Slaves on other plantations who were better fed did not suffer from the disease. Little (1912) mentioned the prevalence of night blindness (nyctalopia, often inaccurately called hemeralopia) among the people of Newfoundland. He also stated that the diet was sufficiently poor to make beri-beri and scurvy of common occurrence. We are informed by Dr. Grenfell that night blindness is common in Labrador. Dr. Appleton, in a private communication, also informs us that she has seen many cases of this eye disease in Newfoundland and Labrador. The diet of the inhabitants of these regions consists almost exclusively of bread made from bolted wheat flour, and canned meats, fish, molasses, tea and small amounts of raisins.

The diet in these regions is of a character which one would expect to be dangerously low in its content of vitamin A as well as deficient in other respects. It is essentially a diet derived from the endosperm of wheat, muscle tissue of beef, pork and fish, and molasses. The last article of food, after its drastic treatment in the manufacture of sugar, would not be expected to have any appreciable value as a source of any vitamin. Such a diet is actually so poor in the vitamins B and C that beri-beri and scurvy are of frequent occurrence.

Dr. Ann Young (personal communication), a medical missionary in India, has informed us that night blindness is common in the vicinity of Calcutta. The popular remedy for the condition consists in poulticing the eyes with an exudate of fresh goat's liver and in including liver in the food. We have also been informed from several sources that the value of liver for the cure of night blindness has been appreciated in Japan from very early times. It has already been pointed out that the glandular organs of animals are good sources of vitamin A. It is not to be wondered at that night blindness in certain parts of the world is in reality due to specific starvation for vitamin A. In Japan where there is no dairy industry, the supply of vitamin A depends largely upon the extent to which leafy vegetables and fish are eaten. Certain edible roots have been shown to contain moderate amounts of this substance, but the amounts of such succulent foods as would ordinarily be eaten by man would scarcely suffice to meet the requirements of the body. The encouragement of the dairy industry in these countries, or the importation of liberal amounts of dried or canned milk, and their wider use in the diet, together with the utilization as adjuvants to the diet of fish

livers and other glandular organs of animals, and of eggs, would go far toward the elimination of night blindness, which, provisionally, may be regarded as a specific syndrome of dietary origin.

From early times records have come down to us showing that epidemics of dropsy have occurred during famines caused by drought or war. In the descriptions of this disorder, it has been noted that hemeralopia frequently preceded the development of the edema. It is said that night blindness is endemic in Russia during the Lenten Fasts. Corneal ulcers and xerosis of the conjunctiva have sometimes been noted, among those who suffered from edema due to faulty nutrition, and ophthalmologists have repeatedly referred these eye changes to poor nourishment. These eye troubles in man result from conditions so complicated that it is not possible without further evidence to attribute all of them to specific starvation for vitamin A. There is, nevertheless, a strong presumption that xerophthalmia or keratomalacia and night blindness (hemeralopia, nyctalopia) not infrequently occur as manifestations of a deficiency of this dietary factor.

This conclusion is supported by the frequent occurrence in the literature of ophthalmology of expressions of the belief that malnutrition may bring about the condition of defective vision known as hemeralopia. Illustrations are found in the association of eye diseases with pellagra by Rampoldi (1885), in the view of Saweljew (1892) that night blindness results from fat hunger, in the report of Roussanow (1885) that cod liver oil is an unfailing remedy for hemeralopia, and in the description of "springtime catarrh" and its associated conjunctivitis by Vetsch (1878). These observations suggest that a diet derived too largely from cereal products, tubers and other foods having similar deficiencies, induces lowering of vitality, which becomes manifested, among other ways, in faulty vision.

Glandular Adipose Tissue.—Cramer (1920) reported a study of the relation of fasting for vitamin A to changes in the histological structure in certain adipose tissue, which he designates as "glandular." Several histologists have recognized that adipose tissue in certain parts of the body, especially the subpleural tissue, the neck and interscapular region, the axillae and around the kidneys, presents a pink and lobulated appearance resembling a fresh pancreas, and differs markedly from ordinary adipose tissue and can be recognized macroscopically. This tissue is histogenetically distinct from ordinary adipose tissue. It has been

described under a variety of names such as "primitive fat organ," "fat gland," "hibernating gland," "brown fat" and "interscapular fat." This fat has in the embryo a characteristic gland-like structure. Certain species, as the rat, domestic mouse and the hibernating animals retain this structure throughout life. In most species, according to Cramer, soon after birth the tissue acquires the appearance of ordinary adipose tissue. This "glandular" adipose tissue is very vascular and is distinct from the ordinary form in its function. Many of its cells are polygonal, present a reticulated appearance, and contain numerous small globules of lipins surrounding a nucleus, in contrast to ordinary fat cells which are oval, and generally contain one large fat globule engorging the cell and pushing the nucleus and protoplasm to one side. The "glandular" adipose tissue appears to contain a larger proportion of anisotropic or doubly refracting lipins than ordinary fatty tissue. The cells of the former are much richer in protoplasm than the common type of adipose cells.

During fasting the "glandular" type tends to persist and shows no obvious diminution, whereas the ordinary type of adipose tissue is rapidly and greatly diminished. In cretins there are circumscribed swellings of the "glandular" adipose tissue in the neck and axillae, and there appears to be a hypertrophy of this tissue when the thyroid gland atrophies, suggesting a functional relationship between them. Cramer points out also that there is evidence of such a relation between the "glandular" adipose tissue and the adrenals. There appears to be a particularly close relationship between the lipins of this tissue and those of the adrenal cortex.

When rats are fed a diet of purified food substances, free from all vitamins, this "glandular" tissue loses all its fat and lipins and presents the appearance of a glandular organ. Under the same conditions there is a great reduction of the lipins of the cortex of the adrenals, but these never become entirely free from them unless death occurs. Under the influence of such a nutritive disturbance the ordinary adipose tissues lose all their fat and other lipins and revert to the connective type of cells. Cramer suggests that the "glandular" adipose tissue is a reservoir of vitamin A, since the disappearance of the characteristic lipins from it and the adrenal cortex is followed by death.

Animals Store Vitamin A.—Sherman and Kramer (1922) state that young rats at weaning time have a store of vitamin A

which promotes growth for a period when they are deprived of this substance. The survival period of full grown rats deprived of vitamin A depends upon the amount available to them before the beginning of the test. Goldblatt and Soames (1923) reported studies on the vitamin A content of the livers of rats on various diets. They found it stored in the liver in proportion to its content in the diet. Half a gram of liver from rats on a vitamin A-free diet that had ceased gaining weight for 3 weeks contained no vitamin A which could be detected. Half a gram of liver from rats on vitamin A-free diet irradiated with the mercury lamp for 4 weeks after they had ceased growing did contain an appreciable amount of the vitamin. The results do not indicate the synthesis of vitamin A by irradiation. Steenbock, Sell and Nelson (1923) examined rat livers from animals on a diet poor in vitamin A and on one containing it in abundance. The young of rats fed 5 per cent of cod liver oil in the stock rations grew almost normally for about 4 months when placed upon a diet very low in vitamin A, whereas young from mothers on the same stock ration without the cod liver oil did not grow at all.

Utilization of Vitamin A Introduced Subcutaneously.—Blegvad (1923) studied the utilization of a vitamin A preparation when injected subcutaneously. He used a preparation believed to be about 100 times as potent as cod liver oil. A man aged 54, suffering from cancer of the liver and jaundice, whose diet had not been deficient in vitamin A, but in whom xerosis of the conjunctivae with severe hemeralopia had developed, was made the subject of an experimental study. He was excreting undigested fat and showed other signs of faulty assimilation and was declining in weight. For 6 weeks the patient was given a diet rich in vitamin A without improvement of the eyes. He was then given every other day a subcutaneous injection of 1 c.c. of the potent preparation. There was no local inflammatory reaction. After 2 injections the xerosis had disappeared and the hemeralopia improved. After 6 injections the patient could see to walk in the evening and read in dim light. He affirms that there are cases of keratomalacia in childhood in which it is impossible to avert permanent blindness by treatment with vitamin-rich foods by mouth, and suggests parenteral administration of vitamin A in such cases. Wollmann and Vagliano (1923) also found that vitamin A has the same effect when given orally or parenterally except that in the latter case its action is delayed by slow absorption.

Pathological Changes Due to Lack of Vitamin A.—For determining the physiological rôle played by a vitamin it is necessary to learn what happens to the cells composing the different tissues in specific starvation for a single one, no other nutritive principle being involved in bringing about metabolic disaster. Osborne and Mendel (1917) found in the urinary tract calculi of calcium phosphate in 91 animals among 857 autopsies. Forty-three per cent of these had not had a satisfactory supply of vitamin A. Mendel (1920) calls attention to the fact that urinary calculi are exceptionally common among peoples of the tropics, and of the Far East, who live on diets quite unlike the mixed diets common among Europeans and Americans. He points out that these calculi are deposited from neutral or alkaline urines, which frequently owe their reaction to the formation of ammonia as a result of bacterial decomposition. The possibility that the invasion of the urinary tract by microorganisms results from the lowered vitality due to partial or complete starvation for vitamin A is suggested by their findings at necropsy. In McCollum and Simmonds' experience calculi have occurred so frequently in animals whose diets contained an abundance of this factor, but were faulty in other respects, that it would seem to be the result of general debility rather than lowered vitality brought about by specific cause. Emmett (1920) confirms the results of others that rats develop xerophthalmia on a vitamin-free diet, and found that boric acid solution and silver protein solutions failed to relieve the condition.

Stephenson and Clark (1920) made bacteriological and histological studies of keratomalacia in rats. Pus from the diseased eyes in many cases showed the presence of a pneumococcus but this was not always present. Organisms of various kinds were found. They believe their results show that when the rat has been on a deficient diet for some time resistance of the cornea is affected, and pathogenic bacteria which by chance are in the conjunctival sac invade the corneal epithelium and destroy the tissue. Wason (1921) studied the pathology of ophthalmia of dietary origin, but was not able to secure any fundamental data concerning the etiology of this condition. She observed in the eyes of rats in which the disease was induced by selective fasting for vitamin A, hyalinization or necrosis of the outer layer of corneal epithelium, exudation of serum and cells into epithelium and stroma, and a proliferation of blood vessels and fibroblasts. In advanced cases invariably the anterior and occasion-

ally the posterior chamber were invaded. She states "the type and virulence of the organisms of secondary infection determine, in part at least, the course of the disease."

Yudkin and Lambert (1922) state that the changes in the eyes resulting from lack of vitamin A do not begin in the cornea but have their origin in the lid. In this respect the sequence of events is the same as that of some of the severer types of acute and chronic conjunctivitis which are frequently complicated by corneal injury with infection and ulceration of this structure. They also say that the lacrymal gland may be the seat of marked pathological changes either degenerative or inflammatory in nature. There was variation in size and form, and in staining properties of the cells which they believe are referable to functional disturbances related to the ophthalmia. Yudkin (1922) describes the incipient changes in the eyes of rats deprived of vitamin A as like those seen in xerosis of the conjunctiva and cornea in man, and that the more advanced picture is that of keratomalacia. Holm (1922) also agrees that the xerophthalmia in rats and infants are identical. Ghose (1922) says that beriberi, scurvy and rickets are all encountered in Bengal but are not widespread. He emphasizes the low resistance of Bengalese to various infectious diseases and that the presence of certain forms of eye diseases suggests that the industrial classes may be living at or below the danger level of vitamin deficiency.

Mori (1922-23) studied histologically the eyes of rats in various stages of specific starvation for vitamin A. He found the first observable change is a tendency for the lacrymal gland to go into a resting stage, and to cease to produce tears. He believes that subsequent changes in the eyes can be accounted for as the result of loss of function of the tear glands. When the supply of tears fails the conjunctival sac is no longer washed continuously as under normal conditions, and bacteria quickly begin to grow there in great numbers. This growth stimulates a migration of leucocytes which accumulate in the eye chamber, causing the well-known hypophyon, which is visible as a yellowness in the pupil. Some of the leucocytes migrate through the outer coating of the eyeball and find their way into the conjunctival sac. Through their dissolution albuminous material accumulates which forms a sticky exudate that tends to paste the eyelids together. The drying of this exudate makes it difficult for the animals to get their eyelids open. It was found possible to cultivate ever increasing numbers of bacteria from swabs taken from

the surface of the eyeball day by day during the progressive fasting for vitamin A.

Owing to dryness of the eye, there is cornification of the external coating, the cells become flattened and pile up in pseudo stratified form, resembling horny layers of skin. Ulcers regularly form on the cornea during the later stages of the disease owing to the death of the tissue. The ulcers finally perforate and the lens pops out. It is possible to induce spectacular recovery in animals whose eyes are in a very damaged condition by administering fats containing an abundance of the vitamin A. Mori regarded the ophthalmia of dietary origin as the analogue in certain respects at least of a human disease common in many parts of the Orient and known as hikan.

Sections of the lids of the eyes of animals suffering from xerophthalmia very often show cystic dilation of the ducts of the Meibomian glands. These cysts are filled with fat—the secretion of the gland cells. The epithelium of the margin of the lid shows evidence of a very marked xerotic process, and the same change is found in the epithelium of the duct. It is very likely that this xerosis may play a part in the occlusion of the duct and the formation of the retention cysts.

The lumina of the acini of the Harderian gland may be either very much dilated and empty, or narrowed by swelling of the secreting cells. Frozen sections of this gland show a remarkable diminution of the fat content of the cells and the lumen. The connective tissue about the gland acini is very often densely infiltrated with round cells.

The mucous cells of the conjunctiva are all entirely destroyed in the course of the xerotic process. It is certain from these findings that the entire secretory apparatus of the eyes of these rats is in a state of dysfunction. The secretion from these glands is either very much diminished or entirely lacking. The changes in the lacrymal gland are the most important in the pathological picture of this disease, and in fact the changes in this gland would seem to be the cause of the lesions in the cornea and conjunctiva. The diminution or the lack of the secretion of the lacrymal gland would explain the dryness of the eyeball as well as the xerosis of the cornea and the conjunctiva. The failure of the lacrymal secretion also explains the increase in number of the organisms found in the conjunctival sac.

Clinically and at autopsy the mucous membrane of the mouths of the rats suffering from xerophthalmia was dry. The animals

thus afflicted in the early stage of the disease sneeze and cough violently but later the cough subsides spontaneously and after a period of dyspnoea the animals die. Nevertheless, histological sections of the mucosa showed no more than the normal cornification of the epithelium. On the other hand, the mucous membrane of the larynx and trachea showed xerotic changes.

Mori also examined the salivary glands of rats suffering from lack of vitamin A, since both in nerve supply and in structure these resemble closely the lacrymal gland. The submaxillary and parotid glands of the rat are serous, the sublingual is a mucous gland. In many rats with xerophthalmia either all or some of these glands are either not secreting at all or secreting very little. The secreting cells become much shrunken, the acini are very small and show no traces of secretion. The epithelium of the intra-lobular ducts is shrunken and the cells are irregular in size.

The epithelium of the principal ducts of these glands often shows cornification and desquamation of the thickened superficial cells, so that the lumen of the duct becomes narrow and is often occluded. Dilation of the small ducts occurs frequently in the parotid gland. Coincident with the xerotic changes and those of the parenchymal cells, the ducts are invaded by bacteria and small abscesses are formed in the gland.

The other secretory organs, such as the liver, pancreas, bowel, kidneys and thyroid, showed no remarkable change except in one case. In one animal the cytoplasm of a small number of cells of the pancreas was very much vacuolated. The reproductive glands of the rat on a vitamin A deficient diet do not function.

Ophthalmia Due to Unbalanced Mineral Content.—McCollum, Simmonds and Becker (1922) made the observation that an ophthalmia indistinguishable in its macroscopic appearance from that produced by deprivation for vitamin A, can be produced in rats receiving an abundance of this vitamin, by including in the diet excessive amounts of certain salts. It did not appear to be due to infection for it appeared in vigorous and rapidly growing rats and not in others that were feeble. The ophthalmia promptly disappeared on substituting more appropriate salt mixtures. Miller (1923) has called attention to a soreness of the eyes in rats kept on a potassium-free diet containing 1 per cent of cod liver oil and no other source of vitamin A. Mori (1923) examined histologically the eyes of rats suffering from "salt ophthalmia" and found that xerophthalmia and keratomalacia were present. He states that the conditions seen in ophthalmia from this cause

are clinically and pathologically identical with those seen in ophthalmia in rats caused by deprivation of vitamin A. He further states that these changes are identical, clinically and pathologically, with human xerophthalmia and keratomalacia.

Transference of Vitamin A in Parabiosis.—Dragstedt and Cooper (1923) report interesting experiments involving parabiosis. Two young rats were united by operation so that they had a common abdominal cavity but no union of blood vessels from one to the other. These animals were kept for several months. When one of such a pair was fed an adequate diet and its mate a diet deficient in vitamin A, the usual symptoms of vitamin A deficiency occurred in the one animal, while its well-fed mate remained normal. They conclude that there is no toxemia in the early stages of the vitamin A deficiency disorder, or if so, the toxins do not pass to the control parabiotic twin in sufficient amounts to induce metabolic disturbances. The vitamin itself cannot pass from one of these to the other.

Blood Pressure in Vitamin Deficiency.—Baldwin, Cook and Nelson (1924) made blood pressure curves from the abdominal aorta in experimental rats suffering from lack of vitamins A and B respectively. Healthy rats maintained a blood pressure of about 84 mm. of Hg. In vitamin A deficient animals there was a slight subnormal pressure, 19 animals giving an average maximum of 75 mm. of Hg. In the B deficient group there was an average maximum of only 34 mm. of Hg.

Vitamin A Deficiency on Fertility in Rats.—Reynolds and Macomber (1921) state that decreasing the content of the diet in vitamin A, in protein or in calcium, markedly decreased the fertility in rats. Evans and Bishop (1922) have described a disturbance of the oestrus in adult female rats when their diet is deficient in vitamin A. These investigators found that there was a prolongation of the oestrus desquamative change in the vaginal epithelium, the smear consisting chiefly, if not exclusively, of cornified cells which in the normal individual characterize the actual period of oestrus and ovulation only. These female rats continue to ovulate and to form corpora lutae, irregularly or at intervals approximating the normal. They also reported on the effects of changes in the proportion of fat, carbohydrate and protein. They state "Animals on carbohydrate-free diets in which protein was neither very high nor low, grew at approximately normal rates; nor was there in either case significant injury to the sex physiology due to carbohydrate absence . . . Much

interest attaches to the physiological effects of the withdrawal of fat. Our study of the incidence of maturity and sequence of estrous cycles shows an evident deletereous effect of the fat-free diets and an effect in excess of that portrayed by growth inadequacy. Nevertheless the ill effect of fat deficiency is least (being almost masked) when protein constitutes 20 or 50 per cent of the diet. When protein is low (12 per cent) it is remarkable that growth on a fat-free diet may be practically normal and yet a depression of the function of the sex glands is very evident. Maturity is postponed and ovulation is far less frequent."

They point out that animals confined to their basal ration of casein, lard, salts and starch, supplemented with yeast and vitamin A, are usually sterile. The animals are not invariably so, but the majority are. Ovulation is unrelated to the reproductive function on this basic diet, since ovulation rate is normal, but there is failure of the females to reproduce. The animals conceive and the placentae are implanted. In other words, the early steps in reproduction are not gravely interfered with, but about the fourteenth day after a positive mating the majority of the females give what Evans and Bishop have designated as the "placental sign." This consists of a slight leakage of blood from the placenta. The vaginal smear contains blood cells due to the leakage of blood from the placenta. Autopsies performed at this time show that the sign has been given by the placenta which are very abnormal and in some instances there has been a complete resorption of the foetuses. They pointed out that when certain natural foods as lettuce are given to a female, even after one or more resorptions have taken place, she will produce normal young.

In summarizing their extensive studies on the production of sterility with nutritional regimens they state that this sterility may be prevented or cured after its appearance in any individual case by the addition of certain natural food-stuffs to the basal ration. They found that lettuce, meat, whole wheat, wheat germ, rolled oats, dried alfalfa and large quantities of milk fat will relieve this condition. Wheat germ oil is said to be especially effective even in very small doses. Whole milk, fresh or dried, cod liver oil, orange juice and yeast, failed to act as curative agents when added to the basal diet. The unknown fertility-conferring factor which they tentatively called X, shown to be present in the above list of foods, cannot be identified with the known vitamins A, B, C or D. Eckstein (1923) found qualitative

underfeeding, viz., lack of fat, vitamin A or phosphorus, produces profound changes in the testes of the male. The organs atrophy, show degenerative changes and fail to perform their function of spermatogenesis. In the female the same conditions did not lead to any essential impairment of function.

Vitamin A Deprivation on Blood-forming Tissues.—Findlay and Mackenzie (1922) state that rats which have survived for a long time on a diet deficient in vitamin A show an almost complete replacement of the hemopoietic tissue by fibrous tissue. There is considerable diminution in leucoblastic cells. If vitamin C is deficient there are patches of gelatinous degeneration and fibrous tissue overgrowth throughout the bone marrow, with disappearance of blood-forming cells. Rats fed a diet containing only one-third of the normal protein but otherwise complete show hemorrhagic areas in the bone marrow with disorganization of hemopoietic cells.

Vitamin A Deficiency on Tumor Growth.—Wyard (1922) reports that in the treatment of malignant disease by a diet free from vitamin A, in no case was any effect of the diet noted on the growth and evolution of the tumor which invariably progressed along the usual lines. Copeman (1923) reports an investigation of the ultimate effect on the adult of a diet deficient in vitamin A. He believes the adult requirements extremely small. He placed patients with cancer on a diet from which foods of animal origin containing the vitamin A were excluded in the hope of obtaining a differential effect on the growing tumor, and believes that this treatment afforded in some cases increased expectation of life and reduced the pain sufficiently to obviate need of anodynes.

Vitamins A and B Excreted in Urine.—Cooper (1922-23) states that ordinary urine of dog or man on their usual diet contains little or no vitamin A, but it was present in both when the diet was rich in vitamin A. Human saliva even on a diet rich in vitamin A contains little or none of it. In dogs the gastric juice from the Pavlov pouch contains vitamin A only when the diet contains it in abundance. Human gastric juice (appetite secretion) contains little vitamin A even when the diet includes eggs and milk. The continuous juice contains little or no vitamin. Pancreatic secretion was lacking in vitamin A. Muckenfuss (1918) and Van der Walle (1922) found vitamin B present in urine.

Vitamin A Deficiency and Intestinal, Aural and Nasal

Florae.—Creekmur (1922) found that in rats fed a diet deficient in vitamin A there was a change in the proportion of gram negative and gram positive bacteria in the feces. The feces of the rats on the deficient diet were dry and hard, which may account for the disappearance of streptococci. There was no change in the proportion of bacteria which fermented glucose, lactose and saccharose. The proportion of H_2S-forming bacteria remained constant. Daniels, Armstrong and Hutton (1923) state that rats fed a diet low in vitamin A may die of bacteriological invasion of ear and nasal cavities before the appearance of xerophthalmia. As the infection advances it leads to an apparent nutritional disaster in which the animal is not restored to a normal condition by feeding rations containing A.

Other Observations on Effects of Deprivation of Vitamin A.—Hintzelmann (1923) reports that animals fed diets deficient in vitamin A suffer atrophy of the endocrine glands. He never observed hypertrophy of the adrenals nor evidence of failure of kidney function. Damianovich and co-workers (1923) state that a lack of vitamin A or B quickly brings on a condition of anaemia which gradually grows more serious. Vitamin B deficiency produces this effect more rapidly and in a more pronounced degree than deficiency in A. The administration of vitamin B ameliorates the condition. Hirai (1923) reports that animals fed a diet deficient in vitamin A for one and one-half months develop marked hyperglucemia. Happ and Wagner (1923) state that in tuberculous children a deficiency of vitamin A has an unfavorable effect upon the disease process, while the abundant administration of this vitamin tends to overcome the tuberculosis. Wengraf and co-workers (1923) say children kept on diets for variable periods of time show that inhibition of growth can be referred to lack of vitamin A. Radiographic studies show that diets which favor growth also favor reparative processes in rickets. In chronic cases rickets is to be considered, they believe, as due to hypovitaminosis rather than to an avitaminosis.

It will be seen from the foregoing that it has been clearly demonstrated that certain fats contain a substance of unknown nature which is one of the nutrients which the diet must furnish if life is to be maintained. The lack of this substance has caused the appearance of an eye disease among peoples in several parts of the world and at various times. It is conclusively shown by experiments on animals that this condition is due to the lack of vitamin A, or its provision in inadequate amounts in the diet.

It does not appear probable that we yet fully understand the changes which take place in the different tissues when they are subjected to deprivation of vitamin A. Such observations as have been recorded have been here brought together.

CHAPTER XVI

STUDIES ON THE PROPERTIES OF VITAMIN A AND THE REQUIREMENTS OF DIFFERENT SPECIES FOR IT

Stability Toward Heat and Chemical Reagents.—In regard to the stability of vitamin A toward heat and reagents, there has existed much difference of opinion. McCollum and Davis (1914) observed that hard boiled egg yolks were still a good source of this vitamin. They also found the melting of butter in a hot water funnel and its filtration during one or two hours, followed by solution in ether, filtration and subsequent evaporation of the ether, did not appreciably lower the content of vitamin A. They, therefore, reported that the substance is fairly stable at high temperatures. They employed in their feeding experiments 3 to 5 per cent of either butter fat or egg yolk fats as the sole source of vitamin A. They concluded from their findings that no extensive destruction could have taken place.

McCollum and Davis (1914) observed that butter fat may be saponified with alcoholic potassium hydroxid and petroleum ether without the destruction of vitamin A. The soaps resulting from this saponification were dissolved in water and shaken with olive oil, samples of which had been shown by previous experiments to be ineffective as a source of vitamin A. The olive oil, which had thus been in intimate contact with the soaps of butter fat, was then removed by ether, and the latter evaporated. This olive oil was found to contain vitamin A, since it caused a marked response with growth in rats which had been denied this dietary essential. This experiment showed that the dietary essential in butter fat is sufficiently stable to withstand saponification in non-aqueous solutions. Steenbock, Sell and Buell (1921) have since recorded their findings that vitamin A resists saponification in the cold by alcoholic potash.

Osborne and Mendel (1915) passed live steam through butter fat for 2½ hours. They found that 18 per cent of this fat in a diet as a source of vitamin A supported normal growth. Mendel has recently stated that butter fat heated for many hours to 96°

C would still suffice as a source of vitamin A when 0.25 gm. per day was included in the diet of the rat. This may be roughly estimated to form about 2.5 per cent of the food mixture. Their data, therefore, support the view that vitamin A is a relatively stable substance at high temperatures.

Steenbock, Boutwell and Kent (1918) took issue with the statements recorded in the preceding paragraph. They stated: "When butter fat is heated for 4 hours at 100°, so much of its vitamin may be destroyed that with our basal rations, using rats as the experimental animal, no demonstrable amounts of the vitamin remained." Steenbock and Boutwell (1920) reported that plant tissues such as yellow maize, chard, carrots, sweet potatoes and squash may be autoclaved at 15 pounds pressure without causing any noticeable destruction of vitamin A.

The explanation of the discrepancy between the results of various investigators in regard to the destruction of vitamin A is afforded by the work of Hopkins (1920). He showed that oxidation is a factor of prime importance in these studies and that in the absence of any possibility of oxidation the vitamin A of butter fat withstands a temperature of 120° for 12 hours without any great loss, whereas aeration for 4 hours at this temperature destroys it almost completely. Drummond and Coward (1920) have confirmed this work and have shown that butter exposed to the air at 37° C gradually loses its vitamin, whereas when protected from the air under similar conditions it retains it. It may be accepted, therefore, that vitamin A is stable at high temperature in the absence of oxygen.

Zilva (1919) found that exposure to a mercury quartz lamp inactivated the vitamin A in butter. Later (1920) he studied the action of ozone on the vitamin A and found that ozone inactivated the vitamin A in fats and oils more rapidly than did oxygen. He concluded that in his previous experiments it was the ozone generated by the lamp which inactivated the butter fat rather than the ultra-violet rays. He found (1922) that the "fat-soluble factor" was destroyed in cod liver oil by exposure to ozone in the dark at ordinary temperature and also by bubbling air through it at 120° C. Spinka (1923) obtained results contrary to those of Zilva with butter fat exposed for 8 hours to the ultra-violet ray. He states that the explanation of Zilva's results is to be found in the production of toxic substances of aldehydic and ketonic nature from butter by the rays. Spinka found that mice fed rayed butter died within 7 to 9 days, whereas

those fed food free from vitamin A lived for about 50 days, after which they developed keratomalacia and died.

Extractability by Solvents.—McCollum, Simmonds and Pitz (1916-17) were the first to study the possibility of extracting vitamin A from plant tissues. It was stated, as the result of experiments with several plant oils, that this substance "is not extracted from plants with the fats by such solvents as ether, chloroform, benzene or acetone, and is therefore not found in any fats or oils of plant origin. Hot alcohol does remove it from plant tissues."

It has been shown by Osborne and Mendel (1918-19) that from certain vegetable materials, especially leafy structures, ether or benzene may extract a certain amount of vitamin A, whereas water is entirely ineffective. Emmett and Luros (1919) showed that benzene or acetone will not extract vitamin A from pancreas, thymus and adrenals. They state that it is safe to say that any of the ether-soluble fat which is present in the desiccated glands has no therapeutic value as a source of vitamin A.

Steenbock and Boutwell (1920) found that when carrots are saturated with lard or corn oil and then extracted with ether, little or none of the vitamin A is removed. The lard preparation contained no vitamin. The corn oil preparations contained it in small but persistent amounts. Ether has little solvent action on vitamin A in carrots. Chloroform and carbon disulphid remove considerable amounts. The vitamin A from alfalfa is very stable and resists saponification with alcoholic potassium hydroxid in the cold. If a saponified alcoholic extract of alfalfa is extracted with ether a mixture containing carotinoids and vitamin A is obtained.

Zilva (1920) demonstrated that alcohol extracts vitamin A from cabbage and carrots. An ethereal extract from the alcoholic fraction equivalent to 25 gm. of fresh carrots caused recovery in rats declining in weight from a deficiency of vitamin A. Cooper (1921) reports that the addition of ether and alcoholic extract of orange peel to a diet otherwise free from vitamin A sufficed for young rats during growth and enabled them to produce young. Similar active preparations were made from lemon and grape fruit peel.

v. Euler (1922) has reported experiments on mice which confirm previous findings that known lipoids, daucosterol, singly or combined, were less effective as a source of vitamin A than a benzene extract of carrot. He (1922) compared a benzene extract

of carrots with a fraction of butter fat obtained by dissolving butter fat in benzene and precipitating with 50 per cent alcohol. These preparations were used singly and together as a source of the vitamin A in experiments with mice. The results are interpreted as indicating that several different components of vitamin A are concerned with growth activity.

Vitamin A and Pigmentation.—A very interesting observation was made by Steenbock (1919). He called attention to the fact that among the vegetable foods the distribution of vitamin A appears to be associated in a remarkable way with yellow pigmentation. Drummond (1919) sought to determine whether vitamin A is one of the yellow pigments of plants. He brought rats into a condition of vitamin A deficiency, and then fed them pure and impure preparations of the pigment carotin. The latter, together with xanthophyll, constitutes the pigments to which the yellow color of many vegetable products are due. The animals did not respond with growth and did not remain free from ophthalmia, which they would have done if a fat containing vitamin A had been administered. The results indicated, therefore, that carotin is not identical with vitamin A.

Palmer (1914) observed that cottonseed oil, when freed from its resinous substances, possesses a fine golden yellow color and is rich in carotinoids, but is devoid of vitamin A. He also showed that the blood of certain species of animals, as sheep, swine, dog, cat, rabbit and guinea pig, is free from carotinoids. This would seem, if the vitamin were one of the pigments, to preclude the entrance of the substance into the tissues of these species. Dolly and Guthrie (1919) found adipose tissues and nerve cells of these species to be free from carotinoid pigments.

Palmer and Kempster (1919) succeeded in growing newly hatched chicks for 6 weeks on a mixture of white maize, white maize meal, white maize bran, skim milk and bone meal. At this time the birds began to fail, but responded at once when pork liver was added to their diet. The latter was shown to be free from carotinoids. At 3 months of age the birds were normal in size. They were then given an occasional feeding of white summer squash and white Spanish onions. There was but a trace of carotinoids in the tissues of the birds when grown, as was shown by the lack of pigmentation of the shanks, ear lobes, beaks and other parts of the body. At the age of 6 months the hens began to lay, and 17 of them produced 893 eggs in 233 days. One hen laid 88 eggs during this period. The yolks were found

to be free from carotinoids. A large number of these eggs were incubated. Viable chicks were hatched, which were normal except for the absence of yellow pigment from the shanks, beaks and other parts.

In the light of these studies Palmer (1919) criticized the view of Steenbock that food-stuffs containing yellow pigment are necessarily rich in vitamin A. He cites several instances in which this relationship does not hold and points especially to vegetable oils. Cottonseed oil has a golden yellow color and is rich in carotinoids and should contain an abundance of fat-soluble vitamin if Steenbock's assumption were true. McCollum, Simmonds and Pitz (1916) found bleached and unbleached cottonseed oil to be free from vitamin A. Drummond and co-workers (1920) tested the hypothesis that there is a relation between yellow pigmentation and content of vitamin A in food-stuffs. They tested twenty-four different fats and oils which were pigmented in varying degrees for their efficiency as sources of this dietary essential, and could discover no relation between vitamin content and color.

Stephenson (1920), working in the laboratory of Hopkins, stated that an impure preparation of carotin, which did not behave like a source of vitamin A when fed as such, acquired the properties of this substance when it was dissolved in palm-kernel oil previously tested and found ineffective for the stimulation of growth. This observation deserves the most careful attention, for if substantiated it would seem to indicate that vitamin A may not be absorbable and utilizable unless it is carried by at least small amounts of fats. She reports also that butter fat can be decolorized of its yellow pigment by absorbing the carotin with charcoal without impairing its value in vitamin A. Stephenson (1920) dissolved 300 gm. of butter fat in 1 liter of light petroleum. This was shaken with 4 gm. of fine birch charcoal for 2 hours, filtered and re-treated with charcoal. A butter fat free from color was obtained. The petroleum was distilled off in vacuo and the resulting butter fat, which was as white as lard, was tested for vitamin A. Growth was maintained during 8 weeks, when the experiment was discontinued. The birch charcoal, therefore, removed the color without removing the vitamin.

Steenbock and Boutwell (1920) had earlier stated that this vitamin is not carotin. In several later papers Steenbock presented data showing the remarkable association of this substance with the yellow pigment of plants. He has shown that when carotin and xanthophyll are separated, vitamin A accompanies

carotin, whereas the xanthophyll fraction is poor in it. Steenbock and co-workers (1921) pointed out that carotin of constant melting point through a number of crystalizations was always found to induce growth in rats after growth had been suspended by lack of vitamin A. They point out, however, that the vitamin is not necessarily a pigment, since the vitamin A content of butters is not of the same order of magnitude as the pigment content, and that cod liver oil containing little pigment may contain much of the vitamin. They studied the stability of vitamin A in extracts of alfalfa, and confirmed the view that it is not necessarily destroyed by saponification. They state that it is not destroyed by oxidation with hydrogen peroxid nor by reduction with nacent hydrogen. Crystalline acetyl derivatives of constituents of the non-saponifiable vitamin fraction of fats were prepared without destroying the vitamin activity.

Palmer and Kennedy (1921) finally disposed of the question by showing that there is no correlation between pigmentation of certain foods and their content of vitamin A. These investigators succeeded in demonstrating that approximately normal growth and reproduction can be secured in the rat on rations free from carotinoid pigments but rich in vitamin A. They first made a critical study of the presence of carotinoid pigments in the albino rat and then instituted growth experiments with this species, using colorless ewe milk fat as the sole source of vitamin A. In other experiments they used, instead of ewe milk fat, colorless egg yolk obtained from hens fed unpigmented food as a source of this factor. The liver, blood, spleen, suprarenals, adipose tissue, ovary and the fat of milk recovered from the stomachs of very young rats were examined with every precaution for thoroughness to determine the presence of carotinoid pigments. The rat milk was absolutely colorless, as was also the fat separated from the adipose tissue. The fat of the blood, suprarenals, spleen and ovaries, and also the liver tissue of new-born rats was entirely devoid of color. The liver of adult rats fed a diet containing an abundance of plant carotinoids yielded an oil which was yellowish, but the pigment did not give any of the tests characteristic of carotin. This substance can not, therefore, be regarded as in any way related to those plant pigments with which vitamin A may be associated.

The milk of the ewe is almost colorless, but gives a very faint test for plant carotinoids. Nine per cent of the diet consisted of this fat, and it was estimated that the ration contained of pig-

ments but 0.127 parts in a million. The diet employed consisted of purified casein, a suitable salt mixture, agar-agar, ewe milk fat, and dextrin carrying the alcoholic extract of ether extracted wheat germ to serve as a source of vitamin B. The latter proved to be an inadequate source of the factor B and was afterwards supplemented by 2 per cent of baker's yeast. On this diet rats grew fairly well for 2½ to 3 months, and 3 females each produced litters of 11 young. Two of these mothers were able to nurse their litters, which were reduced to 6. The young grew essentially normally. On a similar diet in which 15 per cent of colorless egg yolks served as the sole source of vitamin A, 6 rats grew well, 2 increasing in weight from about 70 to about 230 gm. Two females produced each a litter of young which were normally nourished.

Palmer and Kennedy also pointed out certain numerical relations between the carotin content and vitamin A efficiency of various foods. These varied within wide limits. Thus, 15 per cent of their egg yolks containing no carotin were at least equivalent as sources of vitamin A to 85 per cent of yellow maize, although the latter amount furnished 1,400 parts per million of carotin. Five per cent of ewe milk fat they estimated to be equivalent as a source of vitamin A to 1 per cent of dry spinach. The carotin content of one million parts of rations containing these amounts of materials would amount to 0.1073 and 160 parts per million, respectively. The second of these two diets containing about the same amounts of vitamin A would contain about twenty-two hundred times as much pigment as the former. From these results it appears that further attempts to establish a relationship between a yellow pigment and vitamin A are futile.

Palmer (1922) stated that the yellow lipochrome of cod liver oil is not a carotinoid and cannot be removed completely by treatment with charcoal. The yellow pigment in blood serum of pigeons has been identified as xanthophyll. The red pigment in the feet and legs of birds resembles carotin in its solubility but not in other respects. The human organism loses carotinoid pigments by oxidation. Carotinoid skin coloration is due to lowered oxidation processes and superabundance of carotinoids in the diet.

Ghose (1922) found vitamin A in buffalo "ghee" which is pure white. This confirms the observations of Drummond and Coward (1920), who concluded that unless we assume the existence of a

leuco form it does not appear that the vitamin A is a member of the lipochrome group of pigments. They believe the association of vitamin A with yellow pigments is accidental. They studied several samples of butter, cod liver oil, dog body fat, beef fat, mutton fat, pig fat, lard, horse fat, linseed oil, palm, cottonseed, maize, peanut, sesame and olive oils. They point out that the pigments of the cottonseed oil, sesame oil and peanut oil appear to be of a different class. Blackshaw (1923) states that the yellow grains are not rich in vitamin A and that the evidence so far adduced in favor of yellow seeded varieties as regards vitamin A content is not conclusive.

Attempts to Isolate Vitamin A.—Takahashi (1922) described a procedure for saponifying cod liver oil for the preparation of vitamin A. A chloroform solution of his semi-crystalline preparation gives a lipochrome reaction with nitric acid. The substance does not form an acetate and does not absorb bromine but reduces ammonical silver and phosphomolybdate solutions. He found that it is unstable in sunlight and air, taking up oxygen and increasing in weight, with a simultaneous loss in reducing power. He believed it to be an aldehyde.

Takahashi and Kawakami (1923) described the preparation of a semi-crystalline product from cod liver oil which they believe to be nearly pure vitamin A. When 0.08 mg. was administered daily to a mouse dying from lack of vitamin A there was complete recovery in 10 days. They found the substance to contain carbon, hydrogen and oxygen but no nitrogen. It appeared to be of an aldehydic nature. The preparation was insoluble in water but soluble in alcohol, ether, benzene, acetone and other organic solvents. Hattori and Obati (1923) describe several modifications of Takahashi's method for the separation of a concentrated preparation of vitamin A from cod liver oil.

Takahashi (1924), (personal communication), in the laboratory of U. Suzuki, reports the isolation of vitamin A from cod liver oil, butter fat and green laver. He calls the substance biostearin. It has the formula $C_{22}H_{44}O_2$. It is believed to contain two hydroxyl groups, one of which is tertiary, and the other primary or secondary. It is therefore an alcohol closely related to cholesterol. It is believed that cholesterol is a product of biostearin after it has undergone a physiological change in the body. When 0.0001 per cent of biostearin is present in the diet of the rat health and growth are maintained. He states that the effect of biostearin is proportional to its concentration in the diet up to

0.05 per cent, but when this amount is exceeded it has an unfavorable effect.

Takahashi has prepared the benzoate, acetate, hexabromide and ozonide of the substance. It has a molecular weight of about 400.

When a small amount of biostearin was placed in a dish and covered with a dry plate, the image of a screen placed between the plate and the biostearin appears on the plate. This was true even when the plate was at a considerable distance from the biostearin. This happened even though the plate was carefully wrapped in black paper of the kind used to protect dry plates in commerce. When biostearin was sealed in a quartz tube and taken into a dark room, an image of an object between them was produced upon a dry plate, kept near the tube. The action of the biostearin was much less when glass intervened than when quartz was used.

When biostearin was placed in a flask and a current of carbon dioxid was passed over it, and the gas was led out through an outlet before which a dry plate was held, so that the gas which had been in contact with the biostearin was brought into contact with the plate, no action was observed. When a dilute solution of biostearin was spread upon a dry plate and immediately washed off the plate was not affected. When a dry plate was partly immersed to an alcoholic solution of biostearin the submerged part was not affected but the part of the plate outside of the solution was affected. Reduced copper or aluminum foil kept in the same vessel with biostearin was rapidly oxidized. In the environment of biostearin something is formed in the air which gives the KI-starch reaction and the paranitrophenylenediamine reaction. It is believed that it in some manner activates oxygen. Biostearin is a colored substance resembling the carotinoids, but unlike these it does not have a selective absorptive action in the visible part of the spectrum. An alcoholic solution of the substance gave an absorption band near 3200A° of the ultra violet region. The solution becomes fluorescent when illuminated with light from the iron arc.

Origin of Vitamin A in Fish Oils.—Hjort (1914) reported that the food of the cod is not the same at all times. He states that in Norway the early spring rise in diatoms leads to the vast multiplication of copepods which form the food of enormous shoals of small fish which come in to the northern coast (Finmarken) to spawn. These shoals are chiefly composed of capelin

(Mallotus villosus) and they are followed by great numbers of cod, coalfish, haddock and other species which devour them in large quantities. Other species of young fish are frequently consumed by these larger species but their diet also included certain shell fish, larger crustacea, salpa and especially in the case of the cod on the Newfoundland banks, squid.

Lebour (1920) says "One finds that certain copepods and other entomostraca constitute by far the greater part of the food of nearly all very young fish, and that usually each species of fish selects its own favorite food, to which it keeps, indiscriminate feeding seldom or never taking place, and one can usually assign to each fish its own particular food." "Few fish are vegetarians, and it is unusual for any but the youngest fish to eat diatoms." "Very young herring or sprat and a few others often contain green remains which probably belong to some algae, and occasionally diatoms can be recognized in this but even before the yolk sac is absorbed the gut may contain larval molluscs and small crustaceans." Moore (1921) has found that a very rapid multiplication of plant life of the sea takes place in the spring, which he believes to be associated with the increase in light intensity at that period. He says that a short time after the rapid rise in diatoms and other plant organisms there is an associated rise in the number of microscopic animals, particularly copepods. He believes the occurrence of these two related phenomena has an important bearing on the survival and development of young fish hatched at that season.

Coward and Drummond (1921) stated that lower plants (marine algae) containing chlorophyll synthesize vitamin A. Others differently adapted for photosynthesis (red weeds) are not so active in this respect, while those devoid of pigment which play a rôle in carbon assimilation (mushroom) are almost completely deficient. Jameson, Drummond and Coward (1922) reared pure cultures of a common marine diatom (Nitzschia closterium) in sterilized sea water, and found this organism able to synthesize large amounts of vitamin A. They draw a parallel between the dependence of land animals on fresh green leaves and that of marine animals on the synthetic activity of the marine flora for their supplies of vitamin A.

Distribution in Marine Products.—Hjort (1922) found vitamin A in sea weed (green algae, Ulva lactuca and Codium tomentosum), diatom plankton, and the roe of herring and cod. Fresh shrimps (Crangon) and fresh prawns (Pandalus borealis)

contain a growth-promoting vitamin, but it apparently is absent from their benzene extract. Coward and Drummond (1922) studied the content of vitamin of fish ova of brown trout. The eggs are very rich in vitamin A. During the development of the larva fish this substance is utilized so that after the stage at which the contents of the yolk sac are absorbed the supplies are almost exhausted. They suggest that this is a reason why this stage is so critical a period in the development of young fish. If in the post-larval stage or even before the sac is completely absorbed the fish are given vitamin A, growth and development are satisfactory and they appear to store vitamin A in the tissues. On a diet containing adequate protein—freshly ground cod muscle—but deficient in vitamin A no such storage occurs and growth is subnormal. Their experiments confirm previous findings that the stores of vitamin A in the tissues of fish can be derived from the food.

In summing up the studies on the origin of vitamin A, Drummond and Zilva (1922) state that it is now generally agreed that the higher land animals derive their supplies of vitamin A from the green plants either directly or indirectly. The probability of a similar relationship existing in the sea has been suggested by Jameson, Drummond and Coward (1922). There seems to be no adequate grounds for doubting that the ultimate source of vitamin A in the marine oils and liver oils is represented by the marine plants possessing photocatalytic pigments, particularly those which are included in the term "plankton." The periwinkle (Littorina littorea) was interesting as being an example of the direct transference of the vitamin from the algae on which it feeds (Enteromorpha, etc.). The fat extracted from these animals after feeding was deeply colored with chlorophyll and other pigments derived from these green weeds. The dependence of marine animals on certain products of the synthetic powers of marine plants may indeed be as fundamental as that which exists between these two great groups of organisms on land.

Vitamin A in Plant Tissues.—Coward and Drummond (1921) carried out an extensive series of experiments designed to show whether or not germination increases the amount of vitamin A in seeds. They concluded that germination did not appear to increase the vitamin A content nor did they find etiolated seedlings and leaves deficient in chlorophyll to be good sources of it. They found that in the case of sunflower seeds the amount of

vitamin A in the etiolated shoot was not appreciably greater than in the seed itself. Green shoots apparently synthesize large amounts of vitamin A.

Wilson (1922) observed that the synthesis of vitamin A takes place in plants. It has been known for several years that the leaves are rich in vitamin A, whereas the seeds are in general not good sources of it. Wheat was sprouted in the dark and also in sunlight until the sprouts were 2 to 3 inches long. These were cut off, dried and fed (5 per cent) as a source of vitamin A. The results indicate that either etiolated or green wheat sprouts furnish sufficient vitamin A for the growth of white rats. He points out that the proportion of sprouts represents a quantity of seeds which would be an inadequate source of the vitamin. He concluded that vitamin A is produced by the growing plant with or without any accompanying photosynthesis.

Since Wilson and Coward and Drummond obtained such different results with etiolated seedlings it is possible that the different seedlings vary in their power to produce vitamin A.

Coward (1923) studied further the formation of vitamin A in plant tissue. She also studied the relation between the vitamin A and lipochromes in plants. Instances of the association of vitamin A with lipochromes are: tomato pulp, cucumber skin, yellow iris flower, the sepals, petals, petaloid stigmas being used in turn but not the stamens or pollen; orange juice, red capsicum fruit, yellow capsicum fruit, the calyx of the ripened fruit of the winter cherry. Cases where absence of vitamin A is associated with absence of lipochromes are: four varieties of mangolds (the flesh of the root), one variety of swede, the flesh of cucumber, infloresence of the cauliflower, the corollas of ray florets of the Shasta daisy, and the corollas of the ray florets of the purple aster. She states that in the formation of vitamin A some lipochrome is always associated with the vitamin in plant tissues, and where carotin is found, especially when exposed to sunlight, the vitamin may be expected to be present. She concluded (1) that light is necessary for the formation of vitamin A in plant tissues; (2) that this process can be carried on in the absence of CO_2 and of oxygen in the surrounding atmosphere; (3) that it is independent of the presence of chlorophyll in the plant; (4) that it is independent of the ultra violet rays of the spectrum (for such of those as are held back by two plates of window glass) and can be carried on under the influence of the electric light in the absence of sunlight; (5) that the presence of chloroform in

the atmosphere prevents the formation of this vitamin; and (6) that the most complete absence of calcium salts from the nutrient solution in a water culture of Tradescantia does not prevent the formation of vitamin A in the leaves of the plant. Widmark (1924) states that when plants lose the ability to form chlorophyll or lipochrome they also lose their ability to form vitamin A even if kept in full illumination.

Radiant Energy on Growth of Rats and Development of Xerophthalmia.—During the years 1920-1922 there was great interest in the demonstration that ultra violet rays and sun's rays protected both children and animals against rickets. Since rickets had been produced in animals on diets which induced both ophthalmia and rickets, the question arose as to whether the ophthalmia could be prevented by exposing the animals to active radiations. Funk and Sheets (1922) state that there was no difference as regards growth and incidence of xerophthalmia in rats as the result of treatment with ultra violet ray. They (1922) fed rats a diet deficient in vitamin A and exposed them daily 3 to 5 minutes to ultra violet light. They state that the rays prevented rickets and promoted a normal rate of growth but did not prevent xerophthalmia.

Hume (1922) states that irradiation with the quartz mercury vapor lamp can prolong the growth of rats on a diet free or almost free from vitamin A, but attempts to relieve symptoms of vitamin A deficiency of animals which had been on a diet over 90 days failed. She concludes that there is an inter-relation of light and vitamin A but no photosynthesis of the vitamin. Goldblatt and Soames (1922) concluded that rats' growth is stimulated by irradiation with the mercury vapor lamp when the diet is deficient in vitamin A, but state that irradiation is not a substitute for the vitamin but in some way economizes its action. Powers, Park and Simmonds (1923) found that sunlight delays the onset of xerophthalmia in rats but when they are later taken indoors they develop the disease very quickly.

Hume and Smith (1923) claim that rats fed a diet deficient in vitamin A, but kept in glass jars which were exposed to the rays of the mercury vapor lamp for 10 minutes every second day and contained therefore irradiated air grew better than the control animals which were kept under usual laboratory conditions. Rats placed in jars from which irradiated air had been displaced from the quartz lamp and passed through three meters of glass tubing showed poorer growth than control animals. The same

animals subsequently treated in glass jars filled with irradiated air gave some growth response.

Webster and Hill (1923) obtained results contrary to those of Hume and Smith, in that they could not stimulate the growth of rats on diets deficient in vitamin A by irradiation of the air in the jar in which the animals lived by a mercury vapor lamp. They also tried the following procedure without stimulating effect: Ozone, dilute NO_2 gas, air in glass jars which had been exposed to X-ray tubes and which was presumably ionized, and cigarette smoke. Exposure for 30 minutes daily to air led from the enclosure of the mercury vapor lamp failed to influence rickets in young rats on a deficient diet, whereas exposure to the light of the lamp had the well-known curative effect. Goldblatt and Soames (1923) assert that rats on a diet deficient in vitamin A grow normally, their bones develop and calcify provided they are irradiated for about 10 minutes daily by the mercury lamp. When vitamin A is completely absent the rats show improvement on irradiation, but normality is never reached.

Vitamin Requirement of Different Species.—The early studies in nutrition after the formulation of the vitamin hypothesis showed that whereas man, monkey and guinea pig require vitamin C, and suffer from scurvy if deprived of it, other species, as the rat, do not require it, naturally led investigators to inquire concerning the possibility that similar differences in the vitamin requirements of other species might exist. Experimental studies have led to the conclusion that not all species of animals have the same requirements for vitamin A.

Guerrero and Conception (1920) produced xerophthalmia in fowls fed polished rice. They tested tiki-tiki extract and found it to contain vitamin B but not A. Steenbock, Nelson and Hart (1920) found that within a period of 94 days, 3 out of 5 dogs on a diet lacking vitamin A developed ophthalmia. Other dogs given cod liver oil remained healthy. The ophthalmia in one dog was cured by an ether extract of 30 gm. of saponified cod liver oil.

Nelson, Lamb and Heller (1922) fed rabbits a diet deficient in vitamins A and C. A female gave birth to 5 young after 3 months on the diet. Soon afterward she developed xerophthalmia. Two young died before their eyes were opened. A third died when 2 weeks old of ophthalmia. The remaining two showed signs of it. Butter fat was added to the mother's diet. In a few days the eyes of the mother were nearly well. One of the

young was so weak that it died at the end of 3 days. The eyes of the other improved rapidly. It began to grow and doubled its weight in the next 13 days. This is direct evidence that the vitamin A of the food can be transferred promptly to the milk. Their results seem to indicate that the rabbit is immune to scurvy. Boock and Travan (1922) observed keratomalacia in guinea pigs confined to a diet deficient in vitamin A, and were able to cure it by giving cod liver oil. Sugiura and Benedict (1922) drew the conclusion from experiments on pigeons that vitamin A is not essential in any stage of avian nutrition. They also found that birds remain in health on a diet lacking vitamin C.

Emmett and Peacock (1922-23) found young chicks to require the vitamin A, but the young and also the mature pigeon requires but little, if any, of this substance. Neither the chick nor the pigeon requires the vitamin C. They also state that the vitamin B requirement of the rat is less than that of the chick. They confirm the work of Hart and co-workers (1917) that the vitamin A requirement is also less. Birds on a low vitamin A diet showed the accumulation of urates. Their observations are in harmony with those of Beach (1921) in respect to the development of ophthalmia in birds. They also noted leg weakness in chicks on a diet deficient in vitamin A. Plimmer and Rosedale (1923) have confirmed the observation that the bird does not require vitamin C.

Beach (1923) fed fowls a ration which caused the development of a highly pathological condition which would ordinarily be diagnosed as avian diphtheria or roup. The disease was shown not to be infectious and could be controlled by giving foods containing vitamin A. Thus, cod liver oil or green grass served to protect the birds. He described the development of xerophthalmia in poultry as a result of lack of vitamin A, and described the accumulation of crystal urates in the kidneys. The kidneys are usually very pale and are marked with a network of fine lines which are urate-filled tubules. He frequently noted the deposit of white material, probably urates, on the liver and other organs. Hart, Steenbock and co-workers (1924) kept 2 groups of chicks on the same diet except that one had white corn and the other yellow corn, 97 per cent, supplemented with calcium carbonate 2 and sodium chloride 1 per cent. The birds were given skim milk ad libitum. The two groups were exposed to sunlight daily. Those fed white corn (deficient in

vitamin A) did not respond to sunlight treatment. Those receiving yellow corn plus sunlight were beautiful birds. They agree with Emmett and Peacock and disagree with Sugiura and Benedict regarding the vitamin A requirement of birds.

Vitamin A Content of Various Oils.—The fact that fats and oils generally from vegetable substances do not contain the vitamin A, or contain very small amounts of it, and that certain animal fats, *e.g.*, cod liver oil, are very rich in it as compared with others, naturally stimulated much experimental work directed toward comparing quantitatively the content of this substance in various fats and oils from different sources. Osborne and Mendel (1918) were the first to demonstrate that cod liver oil contained vitamin A. McCollum, Simmonds, Shipley and Park (1921) demonstrated in their studies on rickets the existence in cod liver oil of a second vitamin which they called vitamin D. This is still frequently referred to loosely as "a fat-soluble vitamin" or even its effects are attributed by some to vitamin A. The vitamin D, or anti-rachitic principle, is discussed in Chapter XX under rickets.

Zilva and Drummond (1921) found coal fish oil to be less effective than cod liver oil as a source of vitamin A. Pollack oil, although not so active as cod liver oil and coal fish oil, showed a high vitamin A content. Zilva and Miura (1921) found cod liver oil to be many times richer in vitamin A than butter. Drummond and Zilva (1922) described the various methods in effect for the preparation of cod liver oil in Norway. They studied oils prepared by different methods and state that fairly wide variations in vitamin A content in liver oils occur. They believe these variations are the reflection of changes in the diet or in the physiological state of the fish at different seasons.

Zilva and Drummond (1922) found that vitamin A is characteristic of fish oils in general. The soft and hard roe of the cod were rich in it, and it was present in oil of ling, skate, plaice and pollack. They also confirmed its presence in ox liver. Zilva and co-workers (1924) studied the vitamin A content of cod liver oils taken from fish of different ages, and at different times in the sexual cycle, and found no influence of these factors on the quality of the oil. Delf (1924) found among South African oils sesame, mafureir and castor oils are deficient in vitamin A. Seal, whale and blubber oil of the sperm whale all contain it.

Holmes (1922) found pollack liver oil, crude cod liver oil,

pressed cod liver oil, and cod liver oil stearin to contain vitamin A. He (1923) states that liver oils of emaciated cod are highly potent in vitamin A but are not so rich in this principle as the liver oils of plump cod. He (1924) found no difference in vitamin A content in samples of cod liver oil from fresh livers and from livers stored at low temperature out of contact with air for 6 months to one year. He (1924) found 0.8 mg. of crude hake oil to contain sufficient vitamin A to promote growth in rats, and points out that since 1 mg. daily of cod liver oil is adequate for a rat, hake livers do not detract from the quality of cod liver oil.

CHAPTER XVII

THE RELATION OF PELLAGRA TO DIET

Early History.—Pellagra was discovered in northern Spain by Casal in 1735, but for many years it was most common in parts of Italy. Aside from Italy and Spain, pellagra has been prevalent during the last century in parts of France, the Balkans, especially Roumania, and for a lesser time in Egypt. It has, still more recently, afflicted many people in the United States. In this country the disease was not recognized with certainty until 1908, but from that year its incidence rapidly increased until by 1917 there were recorded 170,000 cases of pellagra, principally located in the southern states.

Symptoms.—Pellagra is a disease involving the nervous system, the digestive tract and the skin. Usually one of the first symptoms is soreness and inflammation of the mouth followed by the appearance of remarkably symmetrical erythema on parts of the body. The nervous symptoms are more or less pronounced, and become gradually worse as the disease progresses. The spinal cord is especially the seat of injury, but the central nervous system is also involved in many instances.

Theories as to Its Cause.—Several views have been proposed to explain its etiology. Marzari, more than a century ago, expressed the belief that pellagra resulted from the excessive use of maize as food. Italian investigators later accepted the view that it was caused by eating mouldy maize, the symptoms being caused by a toxic substance produced during the spoilage of the grain. Two other theories have been advanced to account for its etiology: one that it is caused by an infecting organism, and the other, that it is in some way related to faulty diet. None of these views has been established by entirely satisfactory evidence, but the data now point incontrovertibly to a relation between the diet and pellagra. One group of investigators believes that pellagra is an infectious disease, but admits that lowered vitality from faulty nutrition is an important predis-

posing factor. Another group holds that the syndrome results from the lack in the diet of a specific substance or substances, and is, therefore, a deficiency disease. Since almost all students of this disease are agreed that there is some relation between the character of the diet and susceptibility to it, the present chapter will be limited to a discussion of the evidence of the rôle of nutrition as an etiological factor in pellagra. Roussel (1845) stated that the most effective treatment of pellagra is a milk diet. Lussana and Frua (1856) studied the effect of improvement of the diet of pellagrins, and reported that they reduced the mortality in about 8,000 cases, from 24.5 to 4.5 per cent, and that they increased the rate of recovery from 20 to 70 per cent. The idea that the disease could be prevented or cured by proper diet dates back many years.

Wussow and Grindley (1911) emphasized that the diet of the insane patients under their observation was mostly composed of vegetables and that it had a low content of animal protein. At that time the significance of the proper selection of food for the promotion of well-being was not understood.

The Thompson-McFadden Commission, consisting of Siler of the U. S. Army; Garrison of the U. S. Navy, and MacNeal of the New York Post Graduate Medical School (1913), made an epidemiological study of pellagra in Spartanburg County, S. C. They collected a large amount of data regarding the nature of the diets of pellagrins. The information was obtained from statements of physicians, patients, storekeepers, millers, neighbors and others. Their investigations (1914), which were extended to include an examination of the dietaries of non-pellagrins, were interpreted to mean that there is no relation between the character of the diet and the incidence of pellagra. The Commission concluded after several years of observation that "pellagra spread from a pre-existing case as a center in the six villages studied," and that "pellagra morbidity was higher in congested communities using surface privies than in more sparsely settled districts in which similar methods for the disposal of excreta were employed." Further, ". . . the endemic foci of pellagra were located in the districts in which surface privies were in use." In two cotton mill villages completely equipped with a water-carriage system of sewage disposal it was impossible to find cases of pellagra which had certainly originated there, although some cases which had originated elsewhere were present. It was decided that the stable fly (Stomoxys calcitrans) dis-

played certain salient characteristics which seemed to qualify it for the rôle of a transmitter of pellagra.

Voegtlin (1914), in discussing the treatment of pellagra, says that it may be caused by (a) a deficiency or absence of certain "vitamins"; (b) the presence of some toxic substances; (c) a deficiency in certain amino-acids.

Relation of Diet to Pellagra.—Goldberger (1914) studied the factors operating in the etiology of pellagra. His studies have been of the greatest significance in clearing up this problem. He noted that in institutions where pellagra was either epidemic or endemic among the inmates, the physicians, nurses and attendants were almost without exception immune. This indicated that contact with pellagrins or opportunity for transmission by bedbugs did not seem a satisfactory explanation for the transmission of the disease. In institutions where the food purchases were assumed to be alike for patients and attendants, it was found that the diets of the two groups were not comparable. The staff, due to their favorable position, chose the best articles, and diverted certain foods to their own table when the amounts did not suffice for all. The patients were thus at a disadvantage in respect to food. Where the meat and milk supplied for an insane asylum were inadequate, the tendency was for the milk and the desirable cuts of meat to find their way to the table of the staff, while the unattractive pieces of fat or of gristle were served to the patients. Physicians, nurses and attendants were also in a position to supplement the institutional diet.

Goldberger gained the impression after an examination of the character of the dietaries of certain institutions where pellagra was of common occurrence, that more cereals and vegetables were used than in the dietaries of people in better financial circumstances who were practically immune to the disease. This led him to undertake a study of the possibility of preventing the disease in institutions by improving the dietary.

At this time nothing was understood of the peculiar nutritive properties of the different natural food-stuffs. These have become known through the systematic studies of individual foods by means of the biological method introduced by McCollum and Davis (1915). Goldberger compared the diets of persons who developed pellagra with those living in the same region but remaining free from the disease. It was observed that the inhabitants who remained free from the disease took a diet con-

taining more fresh meats, eggs and milk than those among whom the incidence was high. The well-to-do took a better diet throughout the year than did the poorer people, who with the seasons suffered greater changes in their food supply. The winter diet of the latter was simpler and more monotonous. The recognition of the importance of seasonal variations in the diet as an etiological factor in pellagra was one of great importance. It is true that pellagra is in great measure confined to the poorer classes, and afflicts those who live in the less desirable parts of cities and villages. It occurs occasionally among the well-to-do, but much less commonly than among those who depend upon a daily wage. As will be seen later, this can best be accounted for as due to idiosyncrasies which lead the patients to make a faulty selection of foods.

Goldberger, Waring and Willett (1915) studied the conditions which might explain the differences in the incidence of pellagra in two groups of children in an orphanage at Jackson, Miss. In this institution there were children from infancy to 12 years of age. The presence of pellagra was practically confined to the group between the ages of 6 and 12 years. This was correlated with differences in the diets of the two groups with respect to the content of fresh meat "and other animal proteins." The older group were denied these special articles of diet. Analogous conditions were discovered at other institutions.

The earlier studies on beri-beri led Goldberger and his coworkers to reason from analogy that pellagra was in some way either prevented or cured by some specific substance or substances contained in fresh protein foods. With this idea in mind they carried out an experiment with two orphanages and an insane asylum to test whether pellagra could be prevented or cured by proper diet. It was not possible at that time to interpret as satisfactorily as can now be done the nature of the faults in a diet. Goldberger added to the institutional diets liberal amounts of each of the more important articles such as fresh meat, eggs, milk and fresh vegetables, since the institutional diets differed from the better class American diets in the absence of these foods.

In one of the orphanages under observation during 1914, there was no recurrence of pellagra in any of the 67 persons afflicted, who were under surveillance at least until the anniversary of their attack. Among 99 pellagrin residents of this institution who were under observation for at least a year there was not

a single case of the disease after the change in the diet. At the other orphanage 105 pellagrins were observed for at least a year, including the anniversary of their attack, and there was not a single case of recurrence. Sixty-nine individuals who had not suffered from the disease were observed for at least a year and among these not a single case developed.

At the Georgia State Asylum, which was an endemic focus of pellagra, the diet of two wards was modified so as to greatly improve it, but no change was made in hygienic or sanitary conditions. In these two wards 72 patients suffering from pellagra during 1914 were kept under observation at least beyond the anniversary of their last attack, but not one of them showed any signs of recurrence. During the corresponding period 47 per cent of 32 control patients in another ward showed definite recurrences. The conclusion drawn by Goldberger and his associates was that pellagra can be prevented by an appropriate diet, even when the hygienic conditions are unfavorable.

The Prison Farm Experiment.—Goldberger (1915) planned an experiment to determine whether a faulty diet of the type common among pellagrins would produce the disease in man. The plan involved restricting men to a diet similar to that which had been supplied to the institution where pellagra had been endemic, and where it had been relieved by the changes in the food supply described above. This was, indeed, the type of diet characteristic of the homes of the cotton mill workers throughout the section of the South where pellagra was very common. The experiment is of extraordinary interest because of the care with which it was planned and executed, and because of the unquestioned skill of the observers.

A pardon was offered to any of the healthy white men in the state prison who would submit themselves as subjects of experiment. Twelve men offered themselves and of this group 11 actually underwent the test. White male adults were selected because this group of the population in pellagrous districts had shown a lower incidence of the disease than any other race, sex or age. It was believed that the experiment, if it was successful in inducing pellagra in these men, would be the more conclusive because carried out with this selected group of subjects.

The fact that attacks of pellagra occur most frequently in spring and early summer, was taken into account. The experiment was started at a season of the year when, if pellagra was successfully produced after a period corresponding approximately

FIG. 13.—Illustrates the skin lesions on the arm and hand of a pellagrin.—Courtesy of the Thompson Pellagra Commission.

to the winter season, it would appear out of season, *i.e.*, in the autumn. The men were placed on the experimental diet on April 19, 1915, and continued on it until October 31 of the same year.

The experiment was carried out on the prison farm. Of 180 convicts on the farm at the beginning of the experiment, 30 were present until it was terminated. No case of pellagra occurred on the farm during the experiment, or had been noted among the convicts or attendants at the institution in previous years, although it was fairly prevalent in the surrounding country. There would seem to be no question of the fact that the 11 experimental subjects were entirely protected against exposure to pellagra, for they were under guard throughout the experiment.

The diet of the men in the experimental group consisted of white wheat flour, degerminated corn meal (maize), polished rice, starch, sugar, molasses, pork fat, sweet potatoes, collards, turnip greens and coffee. From data furnished by Doctor Goldberger, we calculated that less than 4 per cent of the total energy value of the diet was derived from cabbage, collards, turnip greens and sweet potatoes. The diet was, therefore, essentially derived from degerminated cereals and products made from these, together with molasses and fat pork. It will be recalled that it has been thoroughly demonstrated that such a diet is not adequate for the maintenance of health in adult animals for a very long period. The average protein intake was 41-54 gm.; of fat, 91-134 gm.; and of carbohydrates, between 387 and 513 gm. per man per day. At the end of five and a half months 6 of the 11 men were diagnosed as showing the skin lesions characteristic of incipient pellagra. The diagnosis was concurred in by a number of dermatologists who were familiar with pellagra.

Goldberger and 15 of his associates (1916) next tried in a number of ways to transmit pellagra to themselves by a series of innoculations with blood, nasopharyngeal secretions, feces, urine and desquamating epithelium. The results of this heroic experiment were entirely negative. Not a single one of the volunteers showed any evidence of the disease. These subjects were all presumably nourished on a satisfactory diet previous to and during the experiment. This could be interpreted as evidence that pellagra is a disease of low virulence and that it is liable to attack only that portion of the population with lowered resistance to infection.

Similarities Between Scurvy, Beri-Beri, and Pellagra.—Vedder (1916) has brought together a number of salient facts derived from the observations of several students of pellagra, and interprets them as showing that "there is a certain similarity between pellagra and other known deficiency diseases, namely, beri-beri and scurvy," and "much of the evidence that has been presented as a proof of the infectious nature of pellagra can be reasonably explained in accordance with a deficiency hypothesis." He further concludes: "A deficiency is demonstrable in the diets of most pellagrins. This deficiency appears to me to result from the too exclusive use of wheat flour, in association with corn meal, salt meats and canned goods, goods that are known to be deficient in vitamins."

Vedder has brought out some very important points. "The gastro-intestinal lesions in pellagra and scurvy are analogous." Diarrhea, enteritis, ulceration of the intestines and hemorrhage into the mucous membranes are observed in both conditions. "There are similar nervous symptoms in pellagra and scurvy."

He further points out that suggestive similarities exist between pellagra and beri-beri. The mucous membranes of the stomach and duodenum may be swollen and inflamed, and may present ecchymoses and erosions. "Similarities in the lesions in the nervous system and in the symptomatology referable to the nervous system in pellagra and beri-beri can be distinguished. The pathologic alterations that occur in the cord in pellagra are profound and striking." It has been repeatedly demonstrated that in beri-beri there is degeneration of many of the motor cells in the cord. Roberts (1912) describes the extensive degenerations which have been observed in the spinal cord in pellagra. Vedder states "Now, if we compare this picture with the changes found in the cord in beri-beri, we find that beri-beri is characterized by the same scattered degeneration of the fibers and similar changes in the cells of the cord."

Vedder points out that central neuritis symptoms are very common in pellagrins, and that exactly such changes in the nervous system caused beri-beri for many years to be regarded as an intoxication. "The fact that the spinal fluid in pellagra is normal, points toward deficiency, since it seems improbable that such extensive changes in the spinal cord could occur as the result of an infection without producing corresponding changes in the spinal fluid. Even the skin lesions which are so characteristic of pellagra may be referable to changes in the

cord. Otherwise, how can we explain the marvelous symmetry that is practically the constant characteristic of this symptom?" These are pertinent questions to raise in connection with any decision as to the nature of the factors involved in the etiology of pellagra.

It is evident from the discussion presented above of the conflicting evidence which is presented in the extensive literature relating to pellagra, that it is not at present possible to give credence to all the data which appear to emanate from reliable sources, and to be derived from observations which seem to be accurate, and formulate any satisfactory theory concerning its etiology. Epidemiological, clinical and chemical methods have alike failed to establish beyond question the exact cause of the disease.

Studies of Jobling and Peterson.—Jobling and Peterson (1917) studied the disease in Nashville. They concluded that the disease attacked those who were domiciled in parts of the city which were either without sewage disposal or where it was inadequate. Pellagra could be correlated with unscreened houses, and access of flies to human excreta and to human habitations. They stated that the inhabitants of the South consume excessive amounts of carbohydrates and fats, but pointed out that in Nashville the people of the class in which pellagra occurred most frequently eat during the spring and summer a great deal of potatoes, fruits and other fresh foods. This led them to the belief that pellagra cannot be due to a deficiency of a vitamin. They stated in this connection: "It seems strange, if this theory is correct, that pellagra should be so rare in winter when green foods are scarce, and so frequent in the spring and summer when green foods and fruits are plentiful and cheap." Goldberger believed that the scarcity of fresh fruits and vegetables, especially green foods, in winter, was significant as an argument in favor of the hypothesis that the disease was the result of a faulty diet. It is reasonable to suppose that any effect on health which might follow the adherence to a faulty diet, would, under such circumstances, appear at the end of winter. The effect of a more satisfactory diet in spring and summer would not necessarily become apparent for a time in persons who had been seriously injured by several months restriction to a faulty diet. It may require several months to induce scurvy or beri-beri in man through the agency of a defective diet, and recovery is slow, even when the diet is modified

so as to contain everything which is physiologically essential.

Attempts to Produce Pellagra in Animals.—Chittenden and Underhill (1917) attempted to produce pellagra in dogs by feeding them exclusively on vegetable foods. One diet, consisting of crackers (bolted wheat flour), peas and cotton-seed oil, produced in dogs restricted to it for several months, a condition strikingly suggestive of pellagra in man. The animals developed inflammation of the mouth with sloughing of the mucosa, diarrhea, and skin changes of a nature regarded as analogous to those seen in pellagra.

McCollum and his co-workers had by this time applied the biological method for the analysis of a food-stuff to several important cereals and legume seeds. After the appearance of the paper of Chittenden and Underhill, McCollum, Simmonds and Parsons (1917 and 1918) fed the diet of Chittenden and Underhill supplemented with purified food-stuffs, protein, mineral salts and vitamin A, in various ways to young rats. They also fed the prison diet of Goldberger supplemented by purified proteins, salts and vitamins A and B. The results justified the prediction that these three types of corrections were all that was necessary to make the diet complete for growth in the rat. They were inclined to interpret their results as supporting the view that pellagra is an infectious disease, and that the rôle of diet in its etiology involves only increased susceptibility to infection due to lowered resistance caused by faulty diet persisted in during the winter months. Their results supported the contention of Goldberger that the type of diet which he fed to the prison squad was incomplete, and would lead to loss of vitality, and they made clear the nature of the deficiencies of the prison dietary in so far as they relate to the nutrition of the rat.

Pellagra in Egypt.—The Director of Medical Services of the Egyptian Expeditionary Force directed that a committee of medical men in the British Army make a study of the prevalence of pellagra among Turkish prisoners of war. This report was made by Boyd and Lelean (1919), who concluded that the maximum distribution of pellagra appeared to correspond geographically to the broad belts where maize grew best, and hence formed the larger proportion of food in the classes most pellagrous. They attributed pellagra to starvation for protein, especially to a low intake of protein of low biological value. It was

believed that on this theory the frequent occurrence of pellagra among those who ate mouldy maize could be explained by a lower utilization of the proteins of spoiled grain than of unspoiled. The occurrence of pellagra among those who had never eaten maize could be explained as a consequence of their securing through some dietetic error a protein supply of low biological value.

Bigland (1920) says in relation to the pellagra outbreak in Egypt: "From the experimental side all efforts to obtain organisms from the blood and feces on various media, complement fixation with alcoholic extracts of organs obtained at autopsy, innoculations of rabbits with pellagrous sera and the diagnostic use of maize extract, failed of positive results." An examination of the diets of the pellagrins showed that there was a deficiency in the "biological value of proteins" but there were a few cases in which no such deficiency could be proved. To explain these cases it is assumed that some toxin is formed which acts upon the intestine or its contents in such a way that protein is not properly assimilated.

Chick and Hume (1920) fed monkeys on a low protein diet, the proteins of which were of low biological value. The animals developed skin lesions which resembled pellagra. These were bilaterally symmetrical and appeared to be heightened by direct sunlight as in pellagra. Nervous symptoms were not observed.

Studies of Voegtlin.—Voegtlin (1920) tested on over 100 pellagrous patients the effects of two types of diets on the clinical condition of the disease. Patients with a moderate attack of pellagra uncomplicated by any other disease were placed upon a diet which closely resembled that to which they were accustomed before the attack. This will be designated as diet X. It consisted of the following ingredients:

White bread	300 grams
Butter	30 "
Cabbage	100 "
Corn meal (Maize)	50 "
Ham	25 "
Hominy	75 "
Corn syrup	75 "
Pork	50 "
Potatoes	150 "
Prunes	30 "
Turnip tops	100 "
Sugar	40 "
Milk	40 "

This diet contained 50.5 gm. of protein; 89.8 gm. of fat; 350.8 gm. of carbohydrate; and yielded 2473 calories of energy.

Of the effect of restricting pellagra patients to this diet Voegtlin says, "Almost without exception the general clinical condition of these patients remained either stationary or gradually became more aggravated simultaneously with an increase in the pellagrous manifestations. The skin lesions often spread to parts of the body which had not been affected previously; there was also an increase in the stomatitis and the gastro-intestinal symptoms. The appetite, as a rule, was good for the first few weeks, but diminished gradually. The nervous manifestations, such as disturbances in sensation, reflexes, and mentality, either showed no change or increased in severity. A few cases developed an acute psychosis. A careful examination of the dietary record showed that the patients had consumed sufficient food. The patients were changed after a time to diet Y, the composition of which was given as follows.

Wheat bread	300	grams
Butter	45	"
Corn meal	50	"
Eggs	100	"
Meat	100	"
Orange juice	100	"
Potatoes	150	"
Prunes	30	"
Sugar	40	"
Milk	1000	"

This diet contained 102.9 gm. of protein; 115.3 gm. of fat; 316.2 gm. of carbohydrates; and furnished 2781 calories of energy.

On diet Y, which differed from diet X in containing 4 eggs, 100 gm. of fresh beef and one liter of milk, the patients showed gradual improvement, ending in many cases in the complete disappearance of all recognizable manifestations of the disease. Another group of patients which was placed on diet Y immediately upon their admission to the hospital, showed definite improvement within 2 weeks. Within 2 months the patients were judged to have recovered from the disease, except in a relatively few cases in which it was far advanced. Some of these did not show any benefit from the improved dietary.

Voegtlin next placed patients on diet X for a time until it was observed that their clinical condition remained stationary or grew gradually worse. Some were then given one of the following extracts of natural food-stuffs:

(1) A fat-free, alcoholic extract of yeast or rice polishings.
(2) A fat-free, alcoholic extract of ox liver or thymus gland.

The first of these extracts was chosen because it contained an

abundance of vitamin B. The second contained a relative abundance of vitamins A and B. The results indicated that the administration of extracts of yeast or rice polishings did not modify the course of the disease in patients maintained on diet X, with the possible exception of one case in which certain well-marked nervous symptoms disappeared coincident with the treatment. Voegtlin states that "the administration of the liver preparation to pellagrins was followed by an improvement in their condition, apparently comparable to that produced by the consumption of a diet containing a considerable amount of milk, eggs and meat. The evidence so far available, therefore, indicates that the dietary defect presumably responsible for pellagra is distinctly different from, and probably more complex, than the one causing human beri-beri."

If these results are confirmed by further experiments it is difficult to see any other interpretation which can be placed upon them than that pellagra is a specific deficiency disease, due to the lack of some substance or substances which are not abundant in the articles which entered into the composition of diet X, but were more abundant in milk, eggs and meat, at least in liver. Voegtlin does not imply that pellagra is necessarily due to a deficiency of the diet in a specific substance such as hypothetical pellagra "vitamin," proposed by Funk. He holds rather that the pellagrous syndrome is caused by a combination of the deficiencies in some of the well-recognized food factors.

Voegtlin's reasoning does not seem logical. An alcoholic extract of liver would not improve the inorganic content of diet X, and it is doubtful whether it would improve the biological value of its proteins. The content of diet X in vitamins A, B and C, when compared with ordinary standards, would seem to be moderately satisfactory. Poor quality of protein and relative shortage of the vitamin C were the two apparent deficiencies. The liver extract would contain an abundance of A and B, but would not improve the diet in respect to protein and vitamin C. Diet X, supplemented with purified protein and mineral salts, would be adequate for nutrition of young rats during growth, and for the maintenance of fairly good vigor in the adult for many months. For this species, therefore, there is no necessity for postulating the need of a supplementary addition of a hypothetical anti-pellagra substance. It seems that if liver extracts are so effective in enhancing the diet on which the condition of patients remained stationary, or grew worse, Funk's

hypothesis that pellagra is a specific deficiency disease must be correct. Voegtlin seems illogical in trying to hold simultaneously to two views, viz.: that there is something in an alcoholic extract of fat-free liver which cures pellagra, when it is made to supplement diet X, and also that the syndrome is caused by a combination of the deficiencies in some of the well-recognized food factors.

Pellagra in Infants.—Voegtlin and Harries (1920) reported pellagra in infants which were nursed by pellagrous mothers, and cite older literature which shows that a considerable number of cases of pellagra have been observed in infants. A mother suffering from severe pellagrous lesions was admitted to the hospital while nursing a seven-months-old infant. The child did not show any symptoms of the disease. The mother was placed upon the following diet (Diet A) during her stay of 35 days in the institution. This diet is of a type commonly used by pellagrins.

DIET A

Breakfast.—Hominy, 75 gm.; butter, 15 gm.; corn syrup, 30 gm.; wheat bread, 100 gm.; coffee, with 20 gm. of sugar and 20 c.c. of milk.

Dinner.—Potatoes, 150 gm.; cabbage, 50 gm.; turnip tops, 50 gm.; fat pork, 30 gm.; wheat bread, 100 gm.

Supper.—Corn meal mush, fried in pork fat, 50 gm.; lean boiled ham, 25 gm.; prunes, 30 gm.; wheat bread, 100 gm.; coffee, with 30 gm. of sugar and 20 c.c. of milk.

Samples of milk were secured from this patient, and from four others on the same diet. These were analyzed by the most approved chemical methods for all constituents which can be determined. The lactose, fat, total nitrogen and total solids were found to fall within normal limits, but were below the average. The ash and phosphorus content were normal, the sodium and chlorin contents were above the normal, whereas the calcium, magnesium and potassium were below normal. No tests were applied to show the vitamin content of these milks. The total volume was greatly diminished in many cases.

None of the infants of the 5 pellagrous mothers showed any signs of the disease. It is impossible to correlate the occurrence of pellagra in infants with the character of the milk of pellagrous mothers. It would seem that if any relation existed between the diet of a mother and the disease, the infant suckled

by her should develop pellagra. Pellagra in infants which are nursed by mothers suffering from the disease is by no means common. Mothers usually feed their infants the same food which they eat, when the breast milk is too scanty to suffice for the children. This replacement of a considerable amount of mother's milk by food which leads to pellagra in the adult should, theoretically, predispose the infant to the disease, which does not seem to be the case. No one has been able to demonstrate the presence of anything toxic in the milk or blood of pellagrins.

It is probable that the milk of pellagrous women is deficient in vitamins A, B and C, which are known to produce specific syndromes. The occasional occurrence of pellagra in infants where the mothers are suffering from the disease might seem to be accounted for on the assumption that it is caused by lack of a specific vitamin.

Voegtlin and Harries also report pellagra in an infant about one year of age. Her mother nursed her throughout this time, and showed no evidence of the disease. She was undernourished and suffered from chronic indigestion. No other member of the family showed signs of pellagra. This case would seem to support the view that pellagra is due to an infection with which the child came into contact.

Lustbert and Birchett (1922) report that 3 breast-fed children of pellagrous mothers exhibited certain symptoms of pellagra. The mothers' milk was normal with respect to fat, carbohydrate and protein. The diet of the mother was poor in protein, rich in carbohydrate and fat, consisting chiefly of dried beans, dried peas, corn meal, lard and canned evaporated milk. This diet was deficient in vitamins and in certain inorganic elements. The disease usually was present in the child only in a mild form and responded to dietetic treatment, *i.e.*, removal from the breast and feeding with cow's milk, fruit juices, vegetable purees and albumin milk (for severe cases).

Other Views as to Cause of Pellagra.—Viswalingam (1920) says that the deficiency and parasitic theory holds good in the etiology of pellagra in the Chinese in the Malay States. The freedom of the Malay and the Tamil laborers from the disease is thought to be due chiefly to the better quality of their diet, which includes a variety of fresh vegetables and more or less meat. That faulty diet is not solely responsible for the disease is shown, he believes, by the seasonal recurrence of symptoms in patients placed in a hospital with adequate diets for a con-

siderable time. He states "Whether the infecting agent is an organism which enters the gastro-intestinal system and produces a toxin which gets absorbed into the system and produces the varied symptomatology, or whether owing to a deficiency in the vitamins some deleterious products are created in the intestines and give rise to an intoxication of the system it is difficult to say at present."

Goldberger (1920) shows that the claims of Enright (1920) and Viswalingam (1920) that the food deficiency theory of the causation of pellagra has been exploded are based on insufficient evidence, in that it cannot be assumed in the case of war prisoners that the diet furnished, although adequate, was consumed, because of the racial differences in methods of preparation, seasoning, etc. Bigland's toxin hypothesis appears to be superfluous on the same ground. He also points out (1920) that data relating to diet must be secured from individual pellagrins or non-pellagrins, and not apply merely to the family group. It must be kept in mind that the changes in the diet with the seasons may be of the greatest importance. He also pointed out that the condition which it is necessary for the subject to reach before a diagnosis of pellagra would be made, should be carefully stated. Much of the conflicting data and resulting conflict of views can be explained through failure of investigators to appreciate these facts. It is in part due to the full appreciation of these points by Goldberger, which gives his studies a superior value.

Sullivan (1920) reports that the pellagra-producing diet of the prison farm experiment of Goldberger was lacking in vitamin B, as determined by feeding experiments with hens. Sullivan and Jones (1920) found that the total quantity of protein, carbohydrate, fat and ash available per man per day during a week of the experiment of Goldberger and Wheeler, in which they were able to produce symptoms of pellagra in man was as follows: Protein 40.36 gm., carbohydrate 461.58 gm., fat 108.76 gm., and ash 29.99 gm. The calorific value on this basis would be 3069.5 calories. The sodium chloride added to the food was estimated as 19.61 gm. per man per day.

Goldberger and Tanner (1921) say a diet adequate in mineral elements and known vitamins did not prevent the recurrence of the disease in pellagrins. It is considered that with the exclusion of these two factors the dominating rôle of diet in the causation of pellagra must be referred primarily to the character of

the protein. In preliminary therapeutic trials with amino-acids the dermal lesions in each of two cases seemed to show a favorable reaction to cystine and in a third case the administration of cystine and tryptophan was accompanied by a gain in weight and some improvement in the diarrhea.

Wilson (1921) says pellagra is the ultimate result of a deficient supply of protein. Sufficiency must be judged by the amount available for assimilation, not by the gross protein. Forty grams per day may be taken as the minimum safe value for this factor. Sullivan, Stanton and Dawson (1921) report that casts or albumin or both were found in about 50 per cent of the cases of pellagra, but some severe cases showed no evidence of kidney change. Metabolic experiments indicated poor protein absorption with high intestinal putrefaction. Goldberger (1922) says it appears to be established that the diet controls the course and development of pellagra. The relationship thus disclosed probably depends primarily on a specific quality of the amino-acid make up of the protein supply. Just what amino-acid, or within certain limits combinations of amino-acids, are concerned, remains for future study to determine. The possibility of an unknown factor, conceivably a vitamin alone or in combination with amino-acid deficiency, is not excluded.

Wheeler (1923) emphasizes that in addition to milk, fresh meats often stand out as the controlling factor in the development of pellagra in the individual or household. A prolonged interruption in the quantity of fresh meats consumed in the absence of an adequate supply of milk or other protein foods, frequently precedes the appearance of pellagra. The sensitiveness of borderline dietaries to what would appear on the surface to be a trifling change in some one of the meagerly provided essentials is a point that cannot be over-emphasized in the prevention and control of the disease. He calls attention to several cases where a decrease or cutting off of the milk supply of a household results in the appearance of pellagra.

Intestinal Flora in Pellagra.—Jobling and Arnold (1923) call attention to the fact that Italian investigators have noted changes in the intestinal flora of pellagrins. They observed an increase in hyphomycetes and supposed that these molds were in themselves toxic or produced a toxin when grown on corn. They were never able to identify any of these molds as a causative factor in pellagra. They express the view that the disease would lead one to conclude that pellagra is not a deficiency disease com-

parable to beri-beri and scurvy, but one in which a definite intoxication, arising probably from the intestinal tract, forms the basis of a pathological condition. Jobling and Arnold isolated fungi from the feces in 5 of 9 acute cases of pellagra, and in 2 of 23 so-called chronic cases. These fungi produced fluorescent substances. Examination of the feces of 50 non-pellagrous persons from Nashville and Memphis did not reveal the fungus in a single case. A fluid medium in which the fungus was grown for 2 or 3 weeks became light brown and strongly acid in reaction. When made slightly alkaline it became strongly fluorescent. The fluorescent substance is soluble in lipoid solvents, and can be isolated from the medium by means of ether or ethyl acetate, and removed from the solvent by weak alkalies.

Photodynamic experiments were conducted with the extracts. These were active on a potassium iodid-starch mixture in the light but inactive in the dark. When a series of mice were inoculated, those exposed to the light soon developed edema and reddening of the ears, and edema and swelling of the eyelids. In a few experiments death quickly followed exposure to the sunlight. If inoculations were conducted daily with frequent exposures to light the ears became gangrenous and dropped off. The tail became rough and scaly and there was some loss of hair from the face and head. These lesions are suggestive of the skin changes on the exposed parts of the body in pellagra and the results suggest that pellagra may be due to a sensitization to light through an absorption from the intestinal tract of fluorescent substances produced by an abnormal intestinal flora which persists in the intestinal tract when the diet contains excessive amounts of carbohydrates. Jobling and Arnold do not express a definite belief that this is the etiological factor in pellagra.

Hindhede (1923) asserts that pellagra cannot be due to deficiency in amino-acid supply in the diet. This view he bases upon experimental observations on soldiers and other men who lived for many months on diets which were very poor in protein. His assistant, Madsen, lived (a) for one year on potatoes and margarine; (b) for 6 months on whole rye bread, margarine, prunes, starch and sugar; (c) for 6 months on barley meal porridge made from water, margarine and sugar; and (d) for 2 years on bread, potatoes, cabbage soup and fruit soup without fats. No other investigator has reported observations on human subjects who have remained in a state of health for long periods

on such remarkably simple and monotonous diets, so poorly constituted with respect to several dietary factors. It is difficult to understand, for example, why Madsen did not suffer from scurvy if he actually lived for so long a time on cooked foods only.

Relation of Protein Nutrition to Pellagra.—Goldberger and Tanner (1924) report that in 8 cases of pellagra marked clinical improvement followed the administration of 200 gm. of baked round steak to the diet. In 4 of these the addition of gelatin had previously been of no therapeutic value. In a group of 29 patients in a hospital in a pellagrous district, to whose basal diet 1200 gm. of buttermilk was added, no pellagra developed, although previous experience would have indicated an incidence of 40-50 per cent of pellagra in the time of observation without the milk. One hundred and twenty-five to one hundred and thirty-five grams of butter and 2 gm. per kilogram of cod liver oil daily did not prevent the onset of pellagra. It is believed that "the primary etiological dietary factor in pellagra is a faulty protein (amino-acid) mixture, a deficiency in some as yet unrecognized dietary complex (possibly a vitamin) or some combination of these." There was a marked prevalence of pellagra among Turkish prisoners of war in 1919-1920. It was believed that lack of sufficient protein of good biological value stands in etiological relation to the disease. This is a determining factor. The pellagrin suffers from lack of appetite, deficiency of gastric hydrochloric acid, and hence with accompanying malassimilation of fat, loss of protein through malassimilation, bacterial putrefaction and diarrhea.

From the standpoint of prevention, the researches in this field are of the greatest importance. It seems established that pellagra is in some manner caused by faulty diet, and that the type of diet which is concerned in its etiology is one derived largely from milled cereals, tubers, molasses, syrup and fat pork. It seems proven in numerous cases that milk is the most effective therapeutic agent, and that the inclusion of a liberal amount of milk, meats, eggs and leafy vegetables in the diet will prevent the disease. All authorities are agreed that there is but one effective method of prevention or of treatment for pellagra, viz., a satisfactory diet.

Pellagra afflicts the people of the South largely because they are engaged in the cultivation of a cash crop (cotton), or in its manufacture. Under such conditions the food supply is derived

in great measure throughout the year from the grocery store, and during the winter season, practically exclusively so. The grocer in modern communities restricts his line of foods in great measure to milled cereals and canned goods which are derived either from muscle meats or from seed products (beans, peas, corn, etc.) and molasses. Experiments on animals have shown that it is not possible to prepare from such a list of foods a diet which will promote well-being over a prolonged period. On such a diet ill health and inefficiency are to be expected, and experience abundantly shows that this result is being realized where such dietary practices prevail.

The knowledge of the methods by means of which pellagra can be eradicated is, therefore, quite adequate, and as soon as it can be applied practically the disease may confidently be expected to disappear in proportion to the extent to which reform in the diet of the population can be brought about.

CHAPTER XVIII

IODIN DEFICIENCY AND GOITER

Iodin as Limiting Factor in Nutrition.—It appears to have become established by recent observations that there is danger in certain parts of the United States and elsewhere of human beings and animals failing to secure in their food a sufficient amount of iodin. This element is present in the thyroid gland to the extent of about 0.2 per cent of the dried weight of the gland, or about 10 to 15 mgm. in the entire gland. This element is essential for the formation of an organic iodin-containing compound which acts as a hormone or regulator of metabolism. Kendall (1915) has isolated this substance from the thyroid glands of animals and has given it the name thyroxin. A lack of iodin in the diet prevents the formation of this hormone and leads to hyperplasia and enlargement of the gland.

Salmon and trout, which are reared under hatchery conditions and fed liver and are crowded, and kept in water which is not changed frequently enough, have suffered greatly from goiter. It has been shown by Marine and Lenhart (1910) that treatment of the water in which the fish are kept with potassium iodid causes retrogression of the thyroid and a return to normal. Feeding the fish upon hashed sea fish also led to improvement in the condition of their thyroids, presumably because sea fish contain iodin. They stated that wild fish do not suffer from goiter although pike and bass in Lake Erie frequently suffer from it.

In the Yellowstone river valley there has been for some time a loss of approximately one million young pigs and numerous lambs, calves and colts each year as the result of thyroid disease. This most serious problem in animal production has been studied by Smith (1917), who found that it was only necessary to administer sodium or potassium iodid to the pregnant animals in order to prevent the condition. As the result of the shortage of iodin many of the pigs were born dead, others died within an hour or two, while still others lived 24 to 36 hours. These pigs were born in a hairless condition, and with great enlargement of

the thyroid gland. Hart and Steenbock (1918) have reported results with pigs which are confirmatory of those of Smith.

McCarrison's Views on Goiter.—McCarrison (1922) stated that simple goiter is a deficiency disease due to an insufficient supply of iodin for the needs of the thyroid gland, or more properly for the needs of the body cells, in the form of the gland's iodin-containing hormone. In proof of this definition he gave the following facts: (1) thyroid hyperplasia is readily produced by diets deficient in iodin; (2) iodin in minute doses will prevent and cure goiter when administered at the proper time and season. He stated further: "While theoretically goiter can, and sometimes does, arise in consequence of a deficient supply of iodin in the food, it arises more commonly in spite of the fact that there is present in the food a sufficiency of iodin. There is abundant proof of this statement; it will suffice, however, to mention in this connection the results of one fundamental experiment. If animals be confined in dirty cages they will develop goiter spontaneously, the size of the goiter being dependent on the season of the year, and the duration of exposure to the unhygienic conditions. If, however, they be confined in scrupulously clean cages of the same dimensions goiter will not arise. Since the food supply, and therefore the iodin supply, was the same in both cases, it follows that there are factors provided by unhygienic conditions of life, as in dirty animal cages, which render the available iodin in the food insufficient for the needs of the body in these particular circumstances. A sufficiency may become an insufficiency in the presence of such unhygienic conditions. Further, if an additional supply of iodin be provided, or cod liver oil be administered, goiter will not develop in animals confined in dirty cages." He stated that previous work had demonstrated the importance of gastro-intestinal infection (in which may be included infection of the mouth, nose, tonsils, etc.) as a goiter-producing influence. He concludes the chief known factors, viz., physiological, nutritional, metabolic, toxic, infectious and nervous, may in varying combinations cause goiter. While, therefore, simple goiter is due theoretically to insufficiency of iodin, it is due in practice to a combination of factors which bring out this insufficiency. These views of McCarrison appear to be out of harmony with those of other workers on this problem.

Endemic Goiter Regions.—The most noted of the endemic goiter districts are (1) the Alpine mountain region of Southern

FIG. 14.—A group of dead and hairless pigs. Note the thickness of the necks indicating a goiterous condition. The mother of these pigs had been reared on a high protein and low roughage ration consisting of 15 parts of alfalfa, 25 of corn, 25 of oats, 25 of middlings, and 10 of oil meal, and kept under strict confinement. She produced two litters of hairless pigs before being changed to a ration of 33 parts of clover, 33 parts of corn and 33 of oats, on which the above pigs were produced. (Courtesy of Professor E. B. Hart, Wis. Agr. Exp. Sta.)

FIG. 15.—The same sow that produced the pigs shown in Fig. 14. On the same ration—but fortified with 10 grams of potassium iodid per 100 lbs. of feed a normal litter was produced. (Courtesy of Professor E. B. Hart, Wis. Agr. Exp. Sta.)

Europe, including all Switzerland, Northern France, Northern Italy and the Balkan States; (2) the Himalayan mountain region of Eastern and Southern Asia, the Gilget district of Northern India, and the plateau regions of Western China and Eastern Mongolia; (3) the Andes mountain region of South America, the most noted section of which is the Peruvian plateau; and (4) in North America the entire basin of the Great Lakes, the basin of the St. Lawrence and that portion of the United States and Canada known as the Pacific Northwest. In a well known valley of British Columbia called Pemberton Meadows the incidence of goiter was so great that it was difficult to raise calves, lambs and pigs.

Keith (1924) studied the problem of goiter in the Pemberton Valley in British Columbia. Many of the men, almost every woman and every child born in this district had an enlarged thyroid. Pigs were born hairless, calves were goiterous, mares became goiterous, and many colts were carried beyond full term and showed traces of myxedema. The farm animals were more subject to goiter in late winter and early spring. Many of the eggs of fowls, while fertile, failed to hatch, the apparent reason being a thickening of the white envelope within the shell. Due to the giving of iodin most of these conditions have now disappeared.

Iodin in Drinking Water.—McClendon and Williams (1923) compared the iodin content in drinking water and the distribution of goiter throughout the United States. Division of the country into four zones showed 0.01-0.1 parts per billion of iodin with a rate of 15 to 30 cases of goiter per 1000. With 0.015-1.2 parts per billion of iodin in the water there was a rate of 5-15 cases per 1000. With 0.06-9.0 parts per billion a 1 to 5 rate existed. With 1.4-10.0 parts of iodin per billion there was a rate of 0-1 per 1000.

They (1923) produced enlarged thyroids in rats by giving them a diet very low in iodin. The controls were given one day a week drinking water containing 0.1 per cent of iodin. At the end of 3 months the thyroid gland of the controls weighed from one-half to two-thirds as much as those not receiving iodin.

Little (1923) discusses the iodin treatment of water supplies for the prevention of goiter. The Water Bureau and Health Bureau of Rochester, N. Y., prescribed 0.0001 gm. of iodin taken daily over a period of 15-20 days as a preventive of goiter. Six-

teen pounds of sodium iodid added to the city water supply every day increases the iodin content from one part to twenty parts per billion. This treatment is reported to have given good results.

Ellms (1924) criticizes the treatment of city water supplies with iodin. He points out that it is very expensive and on the average only about 40 per cent efficient. Chlorine applied to the water would tend to liberate iodin from the sodium iodid and the iodin would tend to react with other constituents and possibly affect its therapeutic value.

Treatment of Goiter in School Children.—Marine and Kimball (1917) studied the effects of administering sodium iodid on the incidence and course of goiters in girls in the public schools of Akron, Ohio. In a complete census of the condition of the thyroid gland in the girls from the fifth to the twelfth grades of the school population in this goiterous district, it was found that 1,688 or 43.59 per cent had normal thyroids, 2,184 or 56.41 per cent had enlarged thyroids, and 594 or 13.4 per cent had well-defined, persistent thyroglossal stalks. They administered sodium iodid to a large number of these girls and 6 months later a re-examination of their thyroids was made. The following table shows the results and also the data secured with another group of girls who did not take the prophylactic treatment.

TABLE XVI

Pupils Taking Prophylactic Treatment	*Pupils*	*Per Cent*	*Pupils Not Taking Prophylactic Treatment*	
			Pupils	*Per Cent*
Thyroids remained normal	283	100.0	637	74.0
Increased from normal to slight goiter	0	0.0	259	26.0
Small goiters (unaltered)	287	66.0	759	87.0
Small goiters (disappeared)	141	33.5	10	1.2
Small goiters (increased)	2	0.5	103	11.8
Large goiters (unaltered)	34	66.7	106	95.5
Large goiters (decreased)	17	33.3	5	4.5
Total	764		1,879	
Total number of girls examined	4,415			

These results indicate clearly the value of the administration of suitable doses of iodids at intervals as a means of preventing the hyperplasia of the thyroid gland.

Kimball (1923) found goiter to be at least 6 times more frequent in girls than in boys. He states that a large majority of goiters in boys are congenital and can be prevented by the administration of iodin to the mother during pregnancy. While occasionally a boy does develop goiter at puberty or after illness or as the result of some chronic infection, in most cases the goiter will disappear if it is treated promptly and properly. He believes that the maximum good from a minimal amount of effort would be gained by applying the principle of goiter prevention to girls as a public health measure and that it can best be done through the public school organization. The education on this subject would insure the application of this method in later life.

Olin (1924) made a detailed study of iodin deficiency and the prevalence of goiter in Michigan. In four counties there were 31,612 children examined. Of this number 14,914 had goiters; 40.5 per cent were boys and 53.8 per cent were girls. A method proposed for the elimination of this condition was that of requiring by law that all salt sold for human and animal consumption in goiterous regions shall contain sufficient iodin so that the minimum requirement of 300 mgm. per year would be normally taken by everyone in this area. Dubois (1924) reported a survey of 1805 persons of different ages at Alma, Michigan, for goiter. Of 1400 grade and high school children, 40 per cent were goiterous; of these 41 per cent were boys and 59 per cent girls. In the college 38 per cent were goiterous (44 per cent boys; 56 per cent girls). Wallace reports examination of 69,256 pupils in Utah. 31.4 per cent of boys and 54.2 per cent of girls were affected.

It appears that a lack of iodin leads to a lack of thyroxin, the active principle of the thyroid, because of a lack of the wherewithal for making it. Lack of thyroxin causes enlargement of the thyroid gland in response to under supply of this substance. When the gland has not enlarged, but is starved for iodin, the provision of iodin will prevent goiter. When the gland has already enlarged the giving of iodin may result in the formation of more thyroxin than is necessary, at least for a time. At any rate the gland is stimulated by iodin to produce thyroxin, and so is kept active. It would seem more logical under such con-

ditions to provide thyroxin rather than iodin, for this would rest the gland rather than stimulate functional activity. When there is hyperfunctioning of the thyroid, the provision of liberal amounts of iodin may do distinct harm because of overproduction of the hormone thyroxin which stimulates metabolism to a remarkable degree. It will be apparent, therefore, that the goiter problem is not so simple as many believe it to be. Those with goiters should obtain the advice of a specialist before deciding whether the taking of iodin is a wise plan.

CHAPTER XIX

ABNORMAL SKELETAL DEVELOPMENT IN CHILDREN

Glisson's Studies on Rickets.—In the year 1650 Glisson published the first adequate description of the disease which is still commonly known as rickets. The condition had appeared in the counties of Dorset and Somerset in England about 30 years previous to his writing, and had been called "rickets" by the country people. They derived this word from the Old English "wrikken," which means to bend or twist.

Barlow's Disease.—Barlow (1894) discussed the relationship of infantile scurvy and rickets and expressed his belief that these were true cases of scurvy, due to inadequacy of "living food" reacting on a child with a rickety basis. He described the disease as having a sudden onset at any period after 4 months, but occurring chiefly between the ninth and eighteenth months. The child was generally rickety but was fairly well clothed as far as subcutaneous fat was concerned. From its association with rickets it has become known as scurvy-rickets. The symptoms and signs are well marked, consisting of anaemia, hemorrhage from the gums, extensive subperiosteal hemorrhages, and intense pain in the bones and joints, so that the child fears the slightest movement.

Barlow's disease and rickets are not necessarily diseases of the poor. The former occurs chiefly among children who are well cared for and whose state of nutrition is good so far as fat and weight are concerned. It seldom occurs in breast-fed children, whereas rickets not infrequently occurs in the latter. It chiefly arises in infants of the better class who have been kept rigidly on some proprietary milk food, especially such as are mixed with water or with condensed milk, or who have been kept for a long period on condensed milk alone, or pasteurized or sterilized cow's milk.

Age Incidence.—Rickets appears in infants as early as the third month. Prematurely born children are especially susceptible to the disease. Dunham (1923) reported a case of rickets

which was well developed in a baby 34 days of age. The blood showed a normal calcium but a low inorganic phosphorus content. She believes that this is the earliest age at which rickets has ever been proved to be present. The disease usually manifests itself from the sixth to the twenty-fourth month. Although it is usually a disease of infancy, it sometimes attacks older children and even young adults and is then called late rickets. Children with rickets are as a rule fairly well grown and appear to be fairly well nourished.

Clinical Symptoms.—Rickets is a disease which affects the entire body, although the most noticeable signs of it are seen in the bones. At the beginning of the disease the children are usually constipated. They are restless and irritable, usually apathetic and disinclined to play. They sleep poorly. Frequently a rachitic child rolls its head about on the pillow until the hair is worn off from the back of its head. The child perspires freely, especially about the head.

The muscles are lax and the tendons and ligaments may become elongated. Because of this as well as from the softening of the bones children do not walk or stand at the proper time. Although they have opportunity for exercise they do not profit by it like the normal child. The muscles of the intestines are affected as well as those of the legs and arms. Due to the weakness of these and the muscles of the abdomen a pot belly develops.

As the disease goes on the deformities of the bones begin to show. One of the first to appear is the "rachitic rosary" or a line of knobs on the side of the chest where the bones of the ribs join the cartilages. The walls of the chest are drawn inward every time the child breathes and grooves are formed along either side of the line of attachment of the diaphragm. The chest becomes compressed from side to side and "pigeon breast" deformities develop. Bosses of new bone are formed on the side and front of the skull, and the head acquires a square shape. The ends of the long bones of the extremities become enlarged. The legs become knock-kneed or bowed. The bones of the arms bend, and there is marked enlargement of the epiphyses at the wrists and ankles. In severe cases curvatures of all sorts appear. Some children show very severe anæmia during the course of the disease and some manifest extreme nervousness and even convulsions.

Rickets by itself is seldom fatal, but the child may die from

some complication. Park and Howland (1921) have stated that if a child with rickets does not die during the first year or so from some complication he may improve in health, although the rickets continues and lime salts are not deposited in the bones. The shafts of the ribs and the costo-chondral junctions may acquire ultimately a sufficient strength to withstand the forces exerted upon them. If given sufficient time osteoid tissue will be produced in such quantities both in the cancellous tissue under the periosteum and in the metaphysis itself as to supply the requisite rigidity. Even though the costo-chondral junctions remain weak, respiration can take place in a normal or approximately normal manner provided the shafts of the ribs acquire sufficient strength. Extreme deformities of the thorax are common in children 3 or 4 years of age. While rickets is often looked upon as a disease which in itself does not threaten life, they state that it does threaten life and may be the direct cause of death when it deprives the thorax of its rigidity. It increases the danger to life and respiratory infections, for example, tuberculosis and pneumonia.

Occurrence in Animals.—Hansemann (1906) stated that the successful feeding of a young carnivorous animal during infancy and adolescence was a very difficult matter. He pointed out that in the wild state while still very young they eat glandular organs and chew soft bones, and under these conditions do not develop skeletal abnormalities. When kept in confinement and fed upon muscle meat and body fat, and given a bone so large and hard that little can be gnawed from it, they invariably develop rickets. He also stated that the domestic cat does not develop rickets, whereas the dog does. Because of the tendency of the cat to kill birds, rodents and other small animals and thus supplement its rather restricted diet it escapes rickets. The dog adapts itself more readily to domestication and limits his food to that provided by his owner.

Cheadle and Poynton (1908), in discussing the conditions in the London Zoo, brought out several interesting points in this connection. Those in charge of the zoo had had much difficulty in rearing lion cubs. Young cubs were fed horse flesh and had invariably died with extreme rickets. More than twenty litters had been lost in this way. At the suggestion of Bland-Sutton a litter was given pounded bones, more milk and cod liver oil in addition to the raw meat. The cubs were kept in the same dens with the same amounts of warmth, light and air

as before. The change in the condition of the animals which followed this change in diet was very remarkable. They grew to be strong and healthy.

It is well known that in many of the large zoos of this country there has been great difficulty in rearing young carnivorae. It is now the practice in most cases to supply them with liver, flat bones containing much marrow, together with fat, and at intervals of a few days to give them rabbits or small birds, as pigeons. These are consumed entirely. Confinement does not seriously interfere with their successful development under these conditions of feeding.

Jost and Koch (1914) stated that rickets is a very common disease among pigs, puppies, lambs and kids, but is less common among colts, calves and rabbits. They also pointed out that until recently rickets was extremely common among young carnivorous animals in zoos and circuses, but is uncommon since those in charge have adopted a more rational system of feeding.

Geographical Distribution.—Rickets is most prevalent in children in Great Britain, France, Belgium, Germany, European Russia, Austria, Hungary and the United States of America. Countries in higher and lower latitudes are much less subject to the disease. In Greenland and the Faroe Islands, and in Iceland, it rarely occurs. It is very rare among the Eskimo and Lapps. Hirsch (1885) noted that Finsen in 5 years' practice in Iceland saw only 42 cases and these were of a mild type. In the Scandinavian kingdoms, Norway and Denmark, it has a subordinate place in the statistics of sickness relating to the earliest years of life. Very few cases are found in Southern Italy and the southern provinces of Spain and Greece. Rickets is usually thought to be rare in Turkey but recent observations in Constantinople indicate the reverse. Rickets does not occur among the natives of Africa. It occurred only infrequently in Japan and China until recent years. Due to the various changes in living conditions and dietary habits of the people through contact in commerce, rickets is becoming more prevalent.

Seasonal Variation.—Kassowitz (1884) called attention to the seasonal variation in the incidence of rickets and of tetany. He noted their increase in winter and decline in summer and autumn. Hansemann (1906) pointed out that most children born in the fall and dying in the following spring showed marked rickets, whereas children born in the spring and dying in the fall were almost free from the disease. Schmorl (1909) stated

that rickets may begin at any time, but that the highest percentage of the disease was always found to be between November and May. The percentage of cases with signs of healing increased as the summer progressed, and reached its highest point in the autumn, only to fall again in the winter. Bernhard (1917) and Hess and Unger (1921) have confirmed these observations.

Hess and Unger's Views on Vitamin A Requirement of Children.—Hess and Unger (1919) state that as a result of feeding experiments on animals and clinical reports it is now generally accepted that vitamin A is important for man, and that a lack of it leads to nutritional disorders in children. They reported the results of an experiment designed to answer this question. They placed five infants ranging from 5 to 12 months of age on a diet complete except for its small content of vitamin A. The diet consisted of 180 gm. of skim milk (0.2 per cent of fat), 30 gm. of cane sugar, 15-30 gm. of autolyzed yeast, 15 c.c. of orange juice, 30 gm. of cotton-seed oil and cereal for the older infants. They state that infants did well on this for 8 to 9 months and did not develop rickets. They interpreted their results to mean that a very small amount of this vitamin suffices to meet the needs of human nutrition, or that an insufficient dietary must be continued throughout many months to bring about harmful results. Their views are directly contradictory to those of Mori and of Bloch. Common experience has shown that under the strenuous conditions of food supply to which large groups of people are exposed as a result of war or famine, nutritional defects of a serious character do develop within a short time in children. It is also a matter of common experience that many children develop rickets of great severity when fed a diet having essentially the properties of that described by Hess and Unger. Park (1923) stated "one may state positively that rickets develops on all milk mixtures and on almost all foods or combinations of foods ordinarily given to children."

Incidence of Rickets in American Cities.—Strongman and Bowditch (1921) reported that rickets was becoming alarmingly frequent in the unhygienic quarters of Boston. The Italians were especially affected. The children of the Jews and Poles, although kept under equally unsanitary conditions, were not so susceptible to the disease. The difference they believed to be solely in the diet. The Italians lived mainly on macaroni and bread which are served as a rule in olive or cotton-seed oil. Meat, eggs and fish are too expensive and milk they do not like.

Hess and Unger (1922) reported that it is estimated in large cities that 50-75 per cent of artificially fed children have rickets. Schmorl's data indicated that 96 per cent of children dying under the age of 18 months showed rickets. There were only 7 cases out of 221 that did not show evidences of the disease. Hess and Unger later stated that if careful and refined examinations are made of artificially fed children almost all of them would have a certain degree of rickets.

Hess (1922) found in his experience that about half of the breast-fed infants of a large city showed signs of rickets by the end of March—the peak of the rickets season. He found it to occur in babies whose mothers were well-nourished and had a liberal and varied diet. The conclusion seems warranted that breast milk provides but a scant factor of safety against rickets and that an additional protective influence is needed, viz., light. He pointed out that in London and in New York, the diet of the babies (the milk) is far more adequate and normal than the supply of light. He believes that it was lack of sunlight which brought forth the epidemic of rickets in the large cities of England which led to Glisson's classic investigations in the seventeenth century.

Rarity of Rickets in West of Ireland.—Mellanby (1919) stated that in the west of Ireland, where the death rate is about 30 per 1000, rickets is an unknown disease, whereas in poor urban districts of England where rickets is rife the death rate in children varies from 100 to 300 per 1000. He stated that it is at least suggestive that there may be some relation between rickets and the enormous death rate of towns, even though the disease itself does not kill.

It is of great significance that rickets is absent or nearly so in the west of Ireland. Much of this land is unsuitable for agriculture, and cattle and sheep raising are the important industries. There are few places in the world where the number of cattle in proportion to the population is greater than in Ireland. So far as the authors' investigations have gone the data show that wherever a region is hilly and unfit for farming, or where the soil is so thin and poor that it is suitable only for the grazing of animals (Northern Scotland, the Hebrides, Northern Scandinavia, Western Ireland, Balkan regions, Central Asia and the semi-arid regions of Africa and Asia and Asia Minor) good physical development is characteristic of the people and rickets is unknown or rare among the children. Lack of transportation

facilities which makes it necessary for people to depend upon locally grown food products, and which promotes the home consumption of dairy products, and prevents the introduction of milled cereals, sugar, molasses and the other ordinary food products found in our grocery stores, are factors safeguarding health. In other words, their diet consists of natural foods instead of the refined foods to which we are accustomed.

In cities in or near such regions skeletal defects may be found owing to the similarity of the food supply to that of the people in industrial regions. Where agriculture thrives and the growing of cereal grains and tubers is the most profitable form of agriculture, physical deterioration as shown by stunted growth, physical inferiority and defective and carious teeth, are likely to characterize the people of both the rural districts and of the cities. The prevalence of rickets in most parts of the United States and in the industrial regions of Europe where people are deriving their food supply largely from refined cereal products such as white flour, corn meal, rice, corn grits, tubers, roots, muscle meats and sugar, emphasizes this point.

Mann (1922) has prepared a valuable report of his studies on the occurrence of rickets in London. The observations include housing conditions, income, race, hygienic surroundings and the nutritional factor. He concluded that in London bad housing, overcrowding or deficient air space were not the factors solely responsible for rickets.

Rarity of Rickets in Island of Lewis.—Mellanby (1920-22) gave a most interesting discussion of the living conditions of the people in the Island of Lewis in the Hebrides. These observations are of great interest in illustrating the greater importance of nutrition than of hygienic factors in promoting health, in reducing the infant mortality and in preventng skeletal defects. The diet of the natives of this Island consists in great measure of cod heads stuffed with cod livers, milk, fish, turnips, oat meal and potatoes. Their diet is in great measure a carnivorous one, supplemented with small additions of cereal, tuber and root vegetables. The inhabitants live under very bad hygienic conditions. Many of the homes have a filthy byre in one end of the building. Most of the houses do not contain a window, or if they do it is a fixed one. A peat fire is kept constantly burning, the smoke of which can escape only through the thatched roof or through the open door. The babies are taken out of doors only in bright weather. They are deprived of sunlight most of

the time and breathe an atmosphere sufficiently smoky to make inflamed eyes the rule. Regardless of these unhygienic conditions the children remain practically free from rickets. The death rate of infants in the Islands has not infrequently fallen as low as any place in the British Isles. The influence which counteracts the bad hygienic conditions is the universal practice of breast-feeding. The mothers are taking a diet which, although unattractive to the average European or American, is, when evaluated on the basis of its biological quality as shown by investigations on individual food-stuffs, a highly satisfactory one.

Studies in Inorganic Salt Utilization.—Since the etiology of rickets has long been associated with faulty bones, although the relations of the faulty bones to the mineral metabolism was not understood, it will not be out of place at this point to discuss a few papers directed toward the solution of the problem whether man and animals can make use of inorganic elements for all physiological purposes. Many investigators have desired to learn whether or not a mammal can utilize the inorganic elements essential in nutrition when these are supplied in the form of inorganic or mineral salts such as the chlorids, phosphates and carbonates of the alkali and alkaline earth metals. Experiments by Kunkel (1895) showed clearly that the addition of inorganic salts of iron to the diet of animals fed only milk which is too poor in iron to meet the needs of a growing animal after the store of this element in its body at birth is exhausted, caused the hemoglobin content to rise far above that of animals on the same diet without iron salts. Except in the case of iron it was not possible to study this problem satisfactorily until several things had been learned. It was necessary to have a suitable working hypothesis concerning the simplest list of chemical substances which constitute an adequate diet. The technic of preparing diets of purified food substances so as to induce approximately optimal growth had to be gained from experimental trial and failure. It is not possible, for example, to extract all the phosphorus from natural foods so that only known forms of that element can be added to discover whether they could be utilized for maintaining normal physiological processes. Previous to 1909 one of the questions which occupied the attention of physiological chemists was whether or not inorganic phosphorus could be utilized by an animal for the formation of nucleic acids, lecithin and phosphorized proteins such as casein of milk. McCollum (1909) demonstrated

that inorganic salts of phosphoric acid could be utilized for every physiological purpose. In recent years numerous successful experiments involving growth by Osborne and Mendel (1918) and by McCollum and Simmonds, and others, have repeatedly demonstrated that inorganic compounds of sodium, potassium, calcium, magnesium, chlorin, iodin, phosphorus and iron may be assimilated by animals when no other sources of these elements are provided. The element sulphur must, however, be furnished in the diet in the form of the digestion product of proteins known as cystin.

Utilization of Inorganic Elements from Food-Stuffs.— Courtney, Fales and Bartlett (1917) expressed the view that the practice of adding green vegetables to the diet of infants favorably influenced their metabolism. On the basis of the mineral content they concluded that spinach was the best one to provide a salt addition. Givens and Mendel (1917) found that the calcium in milk was much more effective than soluble calcium lactate in producing calcium retention. McClugage and Mendel (1918) showed that when carrots and spinach were added to diets of cracker meal, meat and lard, the calcium of milk was better utilized than that in calcium carbonate; the calcium in spinach and carrots was poorly assimilated as compared with that in milk. They concluded that green vegetables should not be used extensively as a substitute for milk as a source of calcium.

Sherman (1918) studied many American dietaries and found them to contain less than what he considers to be the optimal amount of calcium, which he stated to be 0.67 gm. per man per day. He accepted 0.45 gm. as a minimum on which a balance could be maintained. He stated that for every 100 gm. of protein the diet should furnish 1.0 gm. of calcium. He calculated the calcium content of 224 presumably typical American dietaries from the records of the United States Department of Agriculture and from those of the New York Association for the Improvement of Conditions Among the Poor. He found the average protein content to be about 1.06 gm. per kilo per man per day, and the calcium content to be about 0.74 gm. He thus found the protein to be 140 per cent and the calcium 64 per cent over the minimum requirement as arrived at by his studies.

Sherman's studies were based upon quantitative observations on the calcium intake and output of human beings over a relatively short period of time, but they doubtless represent essentially the existing conditions among the people studied. They

should not be regarded as proof that the minimum calcium intake found necessary to prevent loss of this element from the body represents the actual need for the optimal well-being or a safe limit with respect to this element. The optimal amount of calcium in the diet of the adult is still unknown, and from such data as are available on the effects of the relation between the amounts and proportions between calcium and phosphorus on metabolism it seems certain that this will be determined in part by the phosphorus and vitamin D content of the diet. There is much reason to believe that any diet will be better for containing more calcium in actual per cent of the element than it does of phosphorus, and this the average American diet does not do unless the calcium-rich foods are abundantly supplied. Hoobler (1920) found that a 7 kgm. infant requires 0.143 to 0.215 gm. of calcium oxid per kilo. One pint of cow's milk furnished nearly one grain of calcium oxid.

Holt and co-workers (1920) found the best absorption of calcium when the latter bore a definite relation to the fat intake. The most favorable relation was 0.045 to 0.060 gm. of calcium oxid per gm. of fat when the intake of fat was ample, *i.e.*, not less than 4 gm. per kilo. They stated that an excessive calcium intake did not increase calcium absorption; the excess was excreted.

Holt, Courtney and Fales (1920) found that human milk contains about one-fifth as much calcium as does cow's milk per fluid volume. These authors stated that the average absorption of calcium oxid by breast-fed infants is 0.06 gm. per kilo of body weight. This amount of calcium oxid equals 0.0428 gm. of calcium. They also found that healthy infants fed cow's milk absorb 0.09 gm. per kilo per day. (This equals 0.0643 gm. of calcium.) They found that the babies at the Babies' Hospital in New York, taking various modifications of cow's milk, absorbed only 45 per cent of their calcium intake. It becomes necessary then to give them a minimal intake of 0.13 gm. of calcium oxid per kilo per day to insure the absorption of the amount corresponding to the low average of breast-fed infants.

Sherman and Hawley (1922) found that on an ordinary mixed diet containing 750 gm. of milk daily and furnishing a total of 0.74 to 1.02 gm. of calcium per day, children 3 to 13 years of age stored 0.15 to 0.62 gm. of calcium per day, the amount being approximately proportional to the size of the child and averaging 0.01 gm. of calcium per kilo of body weight per day. When the

daily allowance of milk was increased to 1000 gm. the storage of calcium was increased. Their results indicated that optimum storage of calcium is made when the diet contains one quart of milk per day for each child. With a normal allowance of other foods this will usually mean a daily intake of at least 1 gm. of calcium for the growing child. They found that children do not seem to utilize the calcium of vegetables as efficiently as they do that of milk. In the experiments reported the calcium balances were more variable and always less favorable when vegetables replaced about half of the milk as a source of calcium. It is possible or even probable that the utilization of calcium is adversely affected in these experiments with vegetables because of a deficiency in the diet of the substance, vitamin D, which influences calcium metabolism.

Blatherwick and Long (1922) found that the calcium and phosphorus derived from vegetables was capable of meeting the maintenance requirements of man. The vegetables used were cabbage, celery, squash, spinach, lettuce and asparagus. Rosemann (1922) described metabolism experiments which proved that 0.715, or in another case 1.43 gm. of calcium oxid taken as calcium chlorid solution daily were entirely resorbed. The chlorin was excreted by the kidneys. He interpreted his experiments to prove that abundant meat eating led to a loss of calcium oxid in the body. The retention of calcium also led to a retention of potassium.

Bogert and co-workers (1922) stated that the addition of yeast to a basal diet composed of lean beef, rice, skim milk, white bread, sugar, corn starch and purified fat from nut margarines, practically free from vitamins, led to a lower excretion of calcium in normal women. The substitution of an equal weight of purified butter fat for the margarin in the vitamin-free diet also resulted in a decreased calcium elimination. Rose (1920) stated that the calcium of carrots was utilized as well as the calcium of milk. Sherman and Hawley (1922) reported that children do not seem to utilize the calcium of vegetables as efficiently as they do that of milk. Blatherwick and Long (1922) found in two experiments with adult women, where about half of the calcium came from an assortment of vegetables, there was a considerable storage, with an intake of 0.5-0.75 gm. of calcium per day. Rose and MacLeod (1923) found with blanched almond containing between 0.132 and 0.223 per cent of calcium, when these contributed about 73 per cent of the total calcium of the

diet, the conditions in the digestive tract may not be quite so favorable for utilization as where carrots or milk form the main source of calcium.

Rickets in Breast-Fed Infants.—A few years ago everyone would have agreed with the statement that a breast-fed child would be satisfactorily fed. The fallacy of this statement is now well known since it has been demonstrated that the diet of the mother determines in great measure the quality of the milk which she is able to produce. It is apparent from the following discussion that the prenatal conditions also determine in great measure the physiological perfection of the young. It emphasizes the soundness of the principle insisted upon by the biologists that all children should have the right to be well born.

Korenchevsky (1922) gives a striking instance of the effect of ante-natal conditions upon the development of rickets in Vienna in 1920 in the experience of Dalyell and Mackay. In a foundling institution in that city two infants were observed, the first born of a healthy mother, and the second of a mother suffering from osteomalacia. The mother of the first acted as foster mother to the second. With the exception of the earlier weeks of life when both infants were nourished solely by their own mothers they received identical diets, consisting of breast milk from the healthy woman supplemented by one or two bottles of cow's milk daily. The children were born in July and August respectively and by the middle of December severe rickets was observed in the child of the mother which had suffered from osteomalacia, although it had been nourished in the same manner as the baby of the healthy woman. The baby from the healthy mother was in good condition. In spite of treatment with cod liver oil the stigma of rickets persisted until the following March when the child was about 9 months old. It seems certain from both human experience and animal experimentation that the young born from a mother whose vitality is depleted may be predisposed to rickets. Although these two children were fed identically the pre-natal history was the determining factor as to their development of rickets.

Relation of Breast-Feeding to Incidence of Rickets.—Rickets was formerly more common among the poorer classes than among the well-to-do. It has always been more prevalent among bottle-fed than among nursed infants. Recently, however, many cases have been reported among breast-fed infants, especially among negro infants. The following table taken from

data collected by Ferguson (1918) shows the relation of rickets in the children of Glasgow to the length of time nursed in months.

TABLE XVII

	Marked Rickets (250 Children)		*Slight Rickets (200 Children)*		*Non-rachitic (200 Children)*	
	No.	*Per Cent*	*No.*	*Per Cent*	*No.*	*Per Cent*
Not at all.....	58	23	32	16	25	12.5
1 month	9	4	6	3	7	3.5
2 months	12	5	5	2.5	3	1.5
3 months	12	5	9	4.5	6	3
4-6 months	17	7	12	6	10	5
7-9 months	17	7	25	12.5	33	16.5
10-12 months ..	54	21	57	28.5	66	33
Over 12 months	70	28	54	27	49	24.5

This table shows that breast feeding is not necessarily satisfactory for an infant. Experimental work in recent years has demonstrated very clearly that the diet of the mother is of fundamental importance in determining her capacity to produce milk of good quality. Ferguson (1918) described two diets which she believed to be typical of what she termed rachitic and non-rachitic families in Glasgow. These are so instructive that they are reproduced here in tabular form.

Average Consumption Per "Man" Per Day of the Chief Articles of Diet in Grams

(A) Rachitic Families. (B) Non-Rachitic Families.

	(*A*)	(*B*)		(*A*)	(*B*)
Flour.....	387.9	376.2	Other cereals.....	15.6	26.9
Potatoes.....	291.0	236.8	Margarine or butter..	32.6	38.5
Milk.....	256.0	309.0	Fish.....	15.7	35.9
Meat.....	89.1	92.6	Eggs.....	15.1	30.4
Sugar.....	91.4	84.0	Cheese.....	6.7	8.2
Oatmeal.....	40.4	36.0			

It is very significant that the quantities of milk, eggs, cheese, fish and butter contained in diet B, over A, were effective in preventing the occurrence of rickets. Ferguson did not consider the

differences in the composition of these types of diets sufficient to support the view that the diet was of importance in the production of rickets. When these diets are evaluated in the light of present day knowledge it is apparent that neither of them is of very good quality for the promotion of growth or for the maintenance of health, although diet B is distinctly better than diet A. Diets such as diet B do not produce optimal nutrition. Children restricted to diet B would also be in a borderline condition of nutritional instability, and the conditions under which one lived would determine whether or not defects definitely attributable to malnutrition would become apparent. Light, exercise and other hygienic factors, as well as occasional opportunities to secure food of special value in a supplementary way, may turn the balance one way or the other. In one case the evidence to the casual observer may appear conclusive that the diet is entirely adequate, whereas in another it may be evident that there is a nutritional defect.

Relation of Diet to Composition of Mother's Milk.—Hess and Unger (1918) studied the diet of the negro women of the Columbus Hill district in New York City. They lived in great measure on milled cereal products, tubers and muscle meats. Nearly all of the children of these mothers developed rickets. They demonstrated that giving cod liver oil to these children prevented the development of rickets. Courtney (1923) discussed the effects of inadequate diets on the inorganic salt content of mother's milk. He stated that the continued use of a deficient diet by the nursing mother may have very serious effects on the child, especially in the later stages of lactation when the mother's reserve has become depleted. He cited several types of diets on which mothers were trying to nurse their children. The following is given as an example of a diet which was common among the women studied: One or two fried eggs, meat (usually beef) three times daily, although occasionally pork chops were eaten; about two slices of bread with no butter, two servings of potato with sauerkraut once a week. There was an entire lack of milk, butter and fresh fruits and vegetables other than potatoes and sauerkraut. Our knowledge of the biological values of food-stuffs makes it clear that a mother could not possibly succeed in nursing her infant on such a food supply.

Gebhart (1924) reported the results of an elaborate attempt made by the New York Association for Improving the Condition of the Poor in trying to induce the mothers to give cod liver oil

to the infants during the three winter months. He believes this resulted in a reduction of at least 70 per cent in the incidence of rickets in a district where it was practically universal. Suzuki (1924) studied the child welfare in Manchuria. He states that it had been thought that rickets was largely found in artificially fed infants, but he found in Dairen that it was more prevalent in the breast-fed infants. He found it very prevalent among the children of the well-to-do classes. He saw many cases of rickets complicated with tetany.

Chick's Observations in Vienna.—Chick and co-workers (1922) reported in their studies in Vienna that rickets developed during the winter and spring in children under hygienic conditions when receiving a diet of fresh milk to which sugar was added. Rickets was prevented in the children by giving cod liver oil, more milk and less carbohydrate. When milk was fed which was low in vitamin A rickets did not develop in the summer. They believe the first six months to be a period of special susceptibility to rickets. They stated that rickets may develop in an infant whose general condition is good or improving and healing of the bones may occur independently of improvement in the general condition. They also stated that rickets was cured by cod liver oil, sunlight or radiation with the mercury vapor lamp. McKay and Mackenzie (1924) discussed the present health of German children. Their observations are parallel with those of Dalyell and Chick in Vienna.

History of Use of Cod Liver Oil.—Guy (1923) gives an interesting history of the use of cod liver oil as a remedy. She found no definite mention of the oil from the various species of Gadus in Greek and Roman literature. Apparently, however, cod liver oil has been used by the fishermen along the coasts of northern Europe for many years. She quotes Bennett that in the island of Shetland and in the north of Scotland generally the liver of the cod has long been considered a special delicacy and hence was given to invalids and people in poor health, particularly for "old pains," and that the fresh oil from the livers was used as butter and was considered especially delicious. She states that the use of cod liver oil by physicians was first reported in England by Darbey at the Manchester Infirmary in 1789. The oil was apparently used mainly as an external remedy for many years. Its recognition as a curative agent in rickets cannot be traced to any person, time or place.

Purdah and Rickets.—Hutchison and Shah (1922) reported

a study of 2,000 children averaging up to 12 years of age who were the sons and daughters of agricultural people, artisans and laborers in and about the town of Nasik, India. Many of these children had a scorbutic taint but few showed evidences of rickets. The statement has been made that rickets is a rare disease in this district. Its existence has been clothed and escaped detection due to the fact that it occurred mostly in well-to-do Hindoo and Mohammedan families. Late rickets has not been recognized by the physicians in this locality. The condition has been treated as malaria or rheumatism under the name of "haddi tap" or bone fever. The high class women live in purdah. This condition they enter at 12 years of age. Newly-born children are not taken out of doors for 3 to 4 months and this period is extended if the child suffers from colds or other illness. When they can walk or otherwise look after themselves they run about like other children but at the age of 12 they go into purdah. This applies to the Mohammedan women of class 1.

The Mohammedan houses have rooms which are very small, the windows are small and high, and the rooms are dark, especially the kitchen. Housing conditions are extremely bad. Privacy is the one thing which is emphasized. It is in these dark houses that the high class Mohammedan women spend their lives except for very short periods of respite.

High caste Hindoo women are not technically in purdah. They go about as they like but are seldom away from their homes more than half an hour daily. A newly-born child is seldom taken out during the first 6 months and not infrequently it is a year old before it is taken out of doors. A newly confined woman must not leave her room for 3 months; the child, being with the mother, does not see light for the first 3 months of its life.

Hutchison and Shah emphasize that in Nasik both the Hindoo and Mohammedan women have two periods in their lives when they are under the influence of purdah, viz., in infancy and after the age of 12 years. In men there is but one period and that is infancy. Lower castes, composed of artisans, laborers and agriculturists, live more in the open air and although their diet is not so satisfactory as the diet of the higher class people the latter are subject to rickets in a more severe form and more universally than are the poorer people, but the children of these also show beading of the ribs and pyorrhea. It is evident that the

diet of the laboring classes is so defective that their outdoor life does not entirely counteract its influence, but serves in some degree to do so.

Osteomalacia.—McCrudden (1910) gives a very interesting paper describing his studies on bone metabolism, especially the pathological process, etiology and treatment of osteomalacia. This work is especially valuable from the historical standpoint. It was written before the recent advances in the field of nutrition, and so does not discuss the disease from the dietary standpoint. In osteomalacia there is softening of the bones and the latter contain much less than the normal amount of calcium salts. It is known to occur in animals in Europe, especially in France where it is called "cachexie ossense" or "maladie des pattes," also in the United States, especially in southern Alabama and in parts of Mississippi and Florida. In these places it occurs especially in oxen, cows, horses, sheep, goats, swine, dogs, rabbits and cats. Tuff (1923) observed osteomalacia frequently in cattle in Norway.

Corson-White (1922) noted the occurrence of osteomalacia in several families of mammals and of birds, and in the Philadelphia zoological gardens this was particularly true among the Cebidae monkeys. A chemical and biological analysis of their diet showed that it was poor in protein, which was also of low biological value, and the diet was also poor in fat. The ash content was low, especially in phosphorus. It was deficient also in vitamins A, B and C, but especially in A.

Seclusion of Women in India and Occurrence of Osteomalacia.—Hutchison and Patel (1921) reported a differential study of the incidence and causes of osteomalacia among the Mohammedans and Hindoos of Bombay. The large preponderance of the disorder among Mohammedan women of the city is correlated with the purdah system which results in a lack of fresh air and exercise. They believed that there was no evidence to show that the cause of osteomalacia resulted from a dietetic deficiency since the disease is not uncommon among the wealthier class of the community. They stated that child marriage and prolonged lactation were important factors, but the sudden onset with fever in many cases suggested an infectious condition.

Osteomalacia in Northern China.—Maxwell and Miles (1924, personal communication) have observed many cases of osteomalacia among women in China, especially in the northern part of the province of Shansi, and in parts of Manchuria. The

diet of the patients consisted essentially of wheat flour, millet, bean flour, Chinese cabbage (Pai ts 'ai), garlic, mustard leaves and salt turnip. Garlic is used as a condiment, and the amount of mustard leaves and salt turnip eaten is small. The diet is, therefore, derived in an extraordinary degree from cereals. Dairy products, meat and eggs are almost entirely lacking.

The disease is especially prevalent among women who have borne children, but makes its appearance frequently among adolescent girls. Cases among males are exceedingly rare. They state, "We can safely say that the food ration of the great majority of the people is deficient both as regards quantity and quality, especially the latter." When given cod liver oil, good food and sunlight the majority of the patients will slowly recover. The usual treatment was with cod liver oil and sunlight, because good food is very expensive.

Their data on the content of calcium and phosphorus in the blood of these patients are extremely interesting. Instead of the calcium content being 10.5-11 mgm. per 100 c.c. of serum, it ranges from 5.2 to 7.4 mgm. The phosphorus of normal serum in the adult is about 3.75 mgm. per 100 c.c. In these patients with osteomalacia it ranged from 1.8 to 3.8 mgm. in 10 patients.

The poverty of these people imposes upon them restrictions of diet which are comparable with those to which we have many times restricted experimental rats. It is of great significance that these subjects who go to extremes in trying to subsist upon a cereal diet suffer the same type of bone changes which are seen in rats on a similar dietary regimen.

The conditions of life of the men and women differ significantly. The former live much out of doors and have better food than the latter, who remain in the house or small courtyard and get little sunlight or exercise. When the women have children they are long confined within doors, as are their infants in the first months of life. The children, therefore, get a poor start because of lack of outdoor life and of poor quality of the breast milk. When osteomalacia begins to affect a woman so that she feels ill she remains indoors away from light and takes less exercise as her condition grows worse. Her habits of life tend to accentuate the severity of her condition. In these women the spine becomes bent and the pelvic bones warp so as to greatly deform them and make child-bearing difficult or impossible unless Caesarian section is resorted to. The long bones are bent and distorted.

Maxwell and Miles state that the poor who are compelled to get out and work in the fields are rarely afflicted with osteomalacia. The rich, who have plenty of nourishing food with meat and fat, are seldom affected. The victims are, they say "what we might almost call the genteel members of society; too proud to work, even if their families would allow it, not rich enough to afford the meat and fat which would correct the deficiencies in other ways." It should be emphasized that meat and fat (cod liver oil excepted) would not serve to improve this diet as would milk and green leafy vegetables, since lack of calcium and a disproportionately high phosphorus content of the diet are the outstanding defects of the diet. Dr. Maxwell informs us that he has never seen a case of xerophthalmia among these people. This is doubtless to be accounted for through the large consumption of millet, which McCollum and Simmonds have shown to be richer in vitamin A than our common American cereals.

The unsatisfactory diet of these Chinese people is the result of unsettled political conditions. They want flocks and herds so as to insure milk and meat, and there is no reason why, if given peace and justice and security of tenure, they should not be able to have these necessities. Brigands despoil the property of the ambitious and destroy initiative. The rational treatment for osteomalacia would be to provide security of life and property so that a high standard of feeding and a more healthful standard of living would prevent its development.

Hunger Osteomalacia in Vienna.—Dalyell and Chick (1921) discussed the occurrence of hunger osteomalacia in Vienna in 1920. They state that the individuals affected had difficulty in walking owing to the pain in the tarsal and metatarsal bones which was generally accompanied by edema of the feet and ankles. The lumbar and sacral regions were also affected and stooping and walking were accompanied with severe pain in these areas of an intense and persistent nature. The thorax was also painful on compression of the ribs. Pain was also felt when breathing or coughing. There was great pain on movement but in cases not completely bed-ridden there was a characteristic stiff waddling gait and the climbing of stairs was especially difficult.

The patient had a characteristic expression, the face was sallow, the eyes sunken and there was an expression of great suffering. The skin was harsh, dry and loose. The food supply of the city diminished progressively during 1917 and onwards. The

diets of the patients examined were usually bread and vegetables with a small amount of sugar and flour. Milk, butter and eggs were unknown; meat was not consumed for the price was prohibitive. The only fat used was a little lard and this was at irregular intervals. It was thought that the disease was not strictly a starvation disease but rather the result of a quantitative deficiency in the diet caused by lack of certain foods. Concurrently with the outbreak of hunger osteomalacia there was an unusual prevalence of rickets among infants and young children, and of late rickets among young adults. Favorable results were obtained in the treatment of both disorders by giving cod liver oil. It was impossible to give a satisfactory diet because of the shortage of foods.

Loll (1923) analyzed the bones of persons suffering from severe "war osteomalacia" in Vienna. He found that the calcium was in every case disproportionately high as compared to the phosphorus. In normal, rachitic and osteomalacia bones the calcium oxid content ranges from 51.8 to 52.10 per cent; the phosphorus pentoxid content from 38.7 to 38.9 per cent. He found in the condition referred to from 56.8 to 76.8 per cent of calcium oxid and phosphorus pentoxid content from 16.2 to 37.3 per cent. In rickets the total ash may sink to 19-34 per cent of the weight of the bone, and in osteomalacia the value may be from 20 to 40 per cent. There was extreme softening of the bones and consequently distortion of the skeletal structures, particularly of the thorax and pelvis. Loll does not give any information concerning the dietaries of the subjects of his studies.

Ash Content of Human Milk.—von Meysenbug (1922) found samples of milk of women whose infants showed no evidence of rickets to contain on an average 4 mgm. of inorganic phosphorus per 100 c.c. of milk. The milk of mothers with rachitic infants contained about the same amount. He concludes from his study that there is no reduction in the inorganic phosphate content of breast milk on which a child develops rickets. Burhans and Smith (1923) examined 54 samples of human milk. They found the calcium and inorganic phosphorus of the milk of the colored race to be smaller than that of the white, whereas the chlorin contents were larger for the colored race. Dubuys and von Meysenbug (1924) made 51 determinations on normal breast milk. They found that the average was 32.6 mgm. of calcium per 100 c.c. of normal breast milk. The extremes were 23.7 and 40.0 mgm. per 100 c.c. The milk from mothers whose children

had rickets averaged 27.5 mgm. with extremes of 18.4 to 39.6 mgm. Grouping the milks according to the age of the child, the calcium was found to diminish as lactation advanced. The milk from colored women was of a lower calcium content than that of white women. The average for white mothers was 31.1 and for colored mothers 27.3 mgm.

Umemori (1924) stated from his study of the occurrence of rickets in breast-fed infants that the mother's milk was slightly low in chlorin, whereas those children suffering from beri-beri were taking milks the chlorin content of which was slightly increased. He found the fat content of mother's milk to be about normal when the children developed rickets.

Calcium Metabolism Studies on Infants.—Telfer (1921) found the retention of calcium and phosphorus in an 8 months old child to be no better on a diet of skim milk and cod liver oil than on a diet of cow's milk to which butter fat was added. Wang, Witt and Felcher (1924) reported that a change from breast milk to cow's milk in infants was invariably accompanied by a change in the quantity of calcium excreted in the feces. The actual quantity excreted was always greater on cow's milk with an average of 0.436 gm. excess over the breast milk. They always used the same child in making these determinations. They found calcium absorption and retention to run parallel, since the calcium output is insignificant. The calcium absorbed and retained is greater when a child is fed cow's milk instead of breast milk, but the percentage utilized and retained is greater for breast milk except for older children. Wang and Davis (1924) reported that a change in diet from breast milk to cow's milk was followed by an increase in the chlorin excretion in both the urine and feces.

Acidosis as Possible Factor in Rickets.—Hodgson (1921) reported that in her experience in many cases of rickets acidosis developed at the height of the disease. Pritchard (1923) stated his belief that the true cause of rickets is probably a relative excess of acid substances produced in the system by a disproportion between the energy intake and output. To quote "I cannot think that we are entitled to describe any case as one of rickets unless the contributory causes have definitely led to such a degree of chronic acidosis or mineral depletion that evidences of it are to be found in soft bones, muscular debility and nervous irritability." Burgess and Osman (1924) reported three cases of acute rickets in which they found a severe acidosis in which acetone bodies played no part. They interpret this to mean that

the acidosis in rickets is due to one or both of the following factors: loss of bases by some other channel, *e.g.*, feces or urine or some other acid substance in the blood. It should be emphasized that although acidosis may play a part in rickets when caused by lack of calcium, excessive phosphorus in proportion to calcium and deficiency of calcifying agencies, it certainly can play no part in inducing the type of rickets which has been most studied histologically in animals, viz., that which is induced by feeding a diet deficient in vitamin D and in phosphorus, and containing an excess of calcium. The latter may be supplied as carbonate, which is a potential neutralizer of acid and under the conditions of the experiments there was a marked excess of base over acid radicals supplied to the body.

In this discussion of the outstanding investigations relating to rickets and related conditions as they occur in different parts of the world we have avoided the introduction of technical details as brought out by the study of this disease as it has been produced experimentally and studied histologically in animals. In connection with the exposition of the principal features of experimental rickets in the forthcoming chapter more detailed evidence as to the curative effects of both cod liver oil and radiant energy will be given.

We have pointed out the rarity of rickets in the Hebrides where the hygienic conditions were bad, but owing to the satisfactory diet of the mothers infants are protected against rickets. The Eskimo children do not develop rickets although they live in dark huts and for about half of the year are in the Arctic twilight. They are breast-fed by mothers who eat large quantities of food rich in vitamin D, and are weaned to a diet containing much of this substance. The Negro and Italian children in American cities are especially susceptible to rickets because the pigments of their skin partially insulate them against the sun's rays. Due to this fact they derive less benefit from the sun than do the fair skinned children. The diets of the Negro and Italian children in American cities are notoriously lacking in those foods which carry vitamin D. The Negro child in Africa does not develop rickets because he lives out of doors and is nursed by a mother who eats food presumably of good quality including leafy vegetables. The striking features in the occurrence and associations of rickets can be explained by the occurrence of radiant energy in the environment or of vitamin D in the food.

CHAPTER XX

EXPERIMENTAL RICKETS

For many years there has been suspected a relationship between faulty diet and the development of rickets. Since softening of the bones is a prominent feature of the disease, lack of sufficient calcium in the diet was early thought to be the cause. Prior to 1918 much research had been directed toward the solution of this problem but the experimental diets were not so planned that the results could be interpreted. A single example of the type of experiment planned for this purpose will serve to illustrate this fact. Dibbelt (1909) sought to produce rickets in dogs by feeding a low calcium diet composed of horse meat, carbohydrates (rice starch) and sodium chlorid. This diet is low in calcium but there are several other factors which contribute to making it a poor diet. It is low in all the known vitamins, it is very high in phosphorus, and aside from the shortage in calcium its inorganic content is not so adjusted that the results could be said to turn on a shortage of this factor. Even if the dogs had developed rickets one could not have said that the condition was due to the absence of calcium although this is the interpretation which would have been put upon the experiment prior to the formation of a satisfactory hypothesis concerning what an adequate diet must contain. The numerous theories which have been previously brought forward to explain the etiology of rickets will not be recounted here, since Dick (1922) and Park (1923) have discussed them in their monographs on this disease.

Rickets and Confinement.—Findlay (1908) reported experiments on the production of rickets in dogs by confinement. He attempted to produce rickets in puppies by feeding diets composed of cereals, or bread and water. The puppies became marantic but did not develop rickets. Control puppies fed a diet of milk and porridge developed rickets with the exception of one animal which was exercised daily in the open air. Due to this fact Findlay's attention became attracted to the possibility

of a connection between rickets and muscular activity. He conducted another series of experiments in which the test animals were kept in cages in the laboratory while the control animals were allowed to run about. The diet was milk and porridge. Eight confined puppies developed rickets, whereas the unconfined ones remained free from the disease. He drew the conclusion that "rickets is due to the want of exercise which invariably goes along with it or is consequent upon confinement." He stated "on clinical and experimental grounds I accordingly conclude that confinement with consequent lack of exercise is the main factor in causing the disease."

Faulty Diet and Bone Growth.—In 1917, while carrying out a series of experiments designed for discovering the nature of the dietary deficiencies of two or more cereal grains, McCollum and Simmonds observed that many of their rats developed beaded ribs and showed collapsed thoraces. A systematic effort was made during the next two years to find what kinds of inorganic salt additions would supplement these cereal diets so as to prevent the abnormalities of the skeleton. It was found that no matter what salts were added a normal skeleton was not produced unless a calcium salt was included in the diet. It was recognized that although these abnormalities suggested rickets, a diagnosis of rickets could not be made from gross bone lesions, but a histo-pathological study was necessary.

In 1919 Dr. John Howland was shown some of these animals. He believed some of these bone conditions were rickets. This interview resulted in a co-operative investigation between the Department of Pediatrics and the Department of Chemical Hygiene, Dr. E. A. Park and Dr. P. G. Shipley representing the former and the authors the latter.

In order to safeguard against error in the interpretation of our experimental results we used several kinds of diets derived from food-stuffs of different origin, since we had had considerable experience in studying the dietary properties of many natural foods. One of the first diets which we used was composed of rolled oats, flaxseed oil meal, sodium chlorid and calcium carbonate in varying amounts. Another diet was composed of four cereal grains, viz., wheat, corn, rolled oats and polished rice, with the two legume seeds, peas and navy beans. Another diet consisted of the cereal grains and legume seeds, together with the addition of casein. These combinations of foods were fed with and without the addition of sodium chlorid and sodium bicar-

bonate. Another diet which we used extensively was composed of beef liver and casein, supplemented with sodium chlorid, potassium chlorid, dextrin and butter fat. It was thought that if we could produce the same histo-pathological changes in the bones on each of these types of diets and could show that they could be prevented by the addition of purified food substances of known character the chances of error in interpreting the cause of rickets would be very small. These diets were modified in many ways by known additions and fed to young rats. Histological examinations were made of the bones and whereas certain of the modifications produced lesions which were characteristic of rickets, as it usually manifests itself in infants, others produced lesions which had some of the characteristics of rickets but differed in some respects from the classical description of the disease as given by Schmorl (1909).

Mellanby's Studies.—While these studies were in progress Mellanby (1919) reported the results of a series of studies on dogs designed to explain the etiology of rickets. He used the following diets.

TABLE XVIII

Rachitic Diets

DIET I	DIET II
Whole milk, 175 c.c. Oatmeal Rice 1–2 gm. NaCl.	Whole milk, 175 c.c. Bread ad lib.
DIET III	**DIET IV**
Separated milk, 175 c.c. Bread (70% wheaten) ad lib. Linseed oil, 10 c.c. Yeast, 10 gm. 1–2 gm. NaCl.	Separated milk, 250-350 c.c. Bread (70% wheaten) ad lib. Linseed oil, 5-15 c.c. Yeast, 5-10 gm. Orange juice, 3 c.c. 1–2 gm. NaCl.

As a result of his studies he stated that the action of fats in the prevention of rickets was graded, certain animal fats being more anti-rachitic than the vegetable fats, and the latter differing from each other greatly. The best of the vegetable fats in preventing rickets he found to be arachis (peanut) and olive oil. He stated that the results seemed to favor the hypothesis that experimental rickets can be prevented by diets containing an abundance of anti-rachitic factor, and that the anti-rachitic

factor and fat-soluble A have somewhat similar distribution. He pointed out that there were several points which were not in harmony with the ordinarily accepted views about vitamin A, since he found fats which do not contain this substance to be effective in preventing rickets. He stated that meat had a stimulating action on the growth of puppies far beyond its fat-soluble A content, and that the anti-rachitic action of meat was greater than the amount of fat-soluble A which it was thought to contain.

He discussed milk as an anti-rachitic food and stated that milk is undoubtedly better than the corresponding amount of butter. Later (1920) he reported further experiments and stated that confinement on an adequate diet will not produce rickets. He stated that freedom in the daytime will not prevent rickets when the diet is inadequate.

Mellanby was apparently unaware of the various factors which are essential in an adequate diet. His experimental work was not planned with a definite understanding of the dietary properties of the food-stuffs which he used. This is evident from statements like the following: "The greater the amount of bread eaten the greater will be the tendency to rickets." "Whether, therefore, the protein effect depends on its own action remains to be decided. If the anti-rachitic effect of protein is established we will be able to comprehend one reason why milk is a better preventive of rickets than the corresponding amount of butter." It will be apparent from the diets Mellanby used that he was feeding in most instances a diet low in calcium but relatively high in phosphorus. The additional calcium added in milk together with the vitamin A aided his animals in making better bone than the addition of vitamin A by itself.

Mellanby was, however, the first to associate the group of fats rich in vitamin A with the prevention of rickets. Whereas some of his data were suggestive that vitamin A was essential for bone growth, some of his results indicated that perhaps another factor was needed which was not identical with vitamin A. His experimental work did not prove or disprove this point. This is true for the reason that in many cases Mellanby did not appreciate the importance of the calcium to phosphorus ratio in his study of rickets. His experiments brought to light the following facts: Cod liver oil possesses great anti-rachitic potency; suet and butter fat were also effective in producing calcification, whereas lard had no anti-rachitic effect; peanut oil was most

FIG. 16.—Illustrates the appearance of the exterior of the thorax of a normal and of a rachitic rat. The animals were the same age. The normal one on the left exhibits the perfection of form in this species. The rickety rat was small, round shouldered, and had the typical "pigeon breast" seen in severe cases of rickets in children. The bending inward of the ribs resulted in a flattening and deformity of the thorax, and the formation of a groove along the line of insertion of the diaphragm.

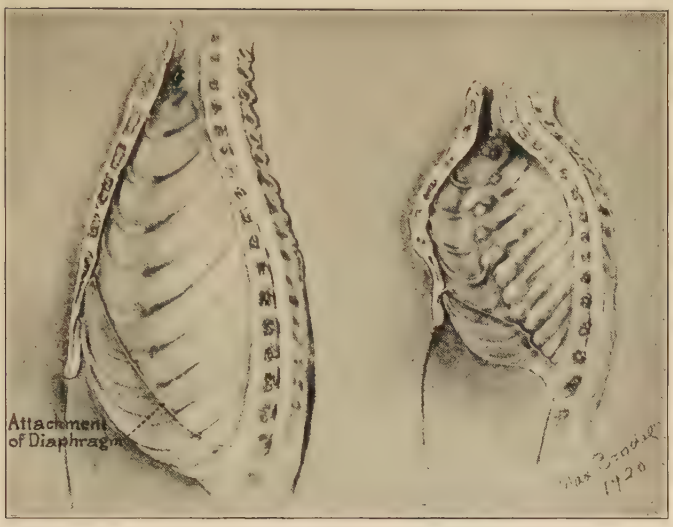


FIG. 17.—Illustrates the appearance of the inside of the thorax of a normal rat and also one suffering from rickets. Note the smooth and symmetrical form of the normal animal on the left. On the right the rickety rat is seen to have great deformity of the thorax. The shoulders are rounded and the breast bone misshapen. There are large knobs on the ribs due to spontaneous fracture and attempt at healing. The junctions of the ribs with the cartilages are enlarged, and the ribs are bent inward at their ventral extremity. The beaded condition of these is the analogue of the "rachitic rosary" seen in children.

effective of the vegetable oils. Cod liver oil was found to be much superior to butter fat in preventing rickets.

Although Mellanby's studies are open to obvious criticism, nevertheless his experiments constituted pioneer work. Since the dietary requirements of the dog are not satisfactorily worked out it is doubtful whether any one could have planned experimental diets for dogs which were as clean cut and decisive as those which have been used with the rat.

Growth of Rats and Rickets.—We found that many of the little rats confined to some of the experimental diets did not grow enough to produce severe rickets. Many of the animals died in a short time because of the pronounced deficiencies of the food supplied. One of the most disturbing problems was the consumption of their fellows which were attacked and killed when the nutrition of a group was faulty. If a rickets-producing diet is one on which the animals do not grow to any extent the condition is less marked, whereas on a diet which induces growth the picture may be exaggerated. One of the greatest problems which had to be met in investigating the etiology of rickets was the ruling out of diets on which the animals did not all perform alike. If the diet induced very little growth in the animals many of the bones presented only a mild grade of rickets, although some would show very pronounced rickets; in other words, the results were inconstant under dietary conditions which produced borderline malnutrition.

Schmorl (1909) showed that the first sign of healing of rickets in rachitic children was the reforming of the provisional zone of calcification on the epiphyseal side of the metaphysis. Howland and Park (1921) proved by means of the X-ray that the administration of cod liver oil to rachitic children was followed by the deposition of lime salts in the cartilage and bones after a period of 15-21 days.

Shipley and co-workers (1921) pointed out that when cod liver oil was administered to animals with rickets a line of calcium phosphate was laid down in the matrix of the proliferative zone. This was observed on diet 2677, which was composed of rolled oats, flaxseed oil meal, sodium chloride and calcium carbonate. It was hoped at the time that this diet could be used to determine the anti-rachitic value of various food-stuffs. It was soon discovered, however, that the animals on this diet or those on diet 2638, which was composed of cereal and legume seeds, did not live sufficiently long or grow enough to produce a wide rachitic

metaphysis which was free from calcified spicules. On these diets animals frequently killed and ate their companions which also produced a line of healing. This led to confusion. The value of this reaction is dependent upon the certainty with which a given diet will produce bones in which the epiphyseal cartilage and the metaphysis are calcium-free. In other words, it is not sufficient that the diet shall produce definite rickets; it must cause an exaggerated form of florid rickets to develop without fail. One of the problems which we undertook to solve was to plan a diet which, without containing an anti-rachitic fat, would not induce ophthalmia and which would induce sufficient growth in the animal in order to give a wide metaphysis which would have no signs of healing in it.

Sequence of Events in Growth of Bone.—It will not be out of place at this point to discuss the sequence of events in the growth of a normal bone, and also the abnormal histological changes in rachitic bones in order to give a clearer idea of the points which it is intended to discuss. The growth of a bone in length is due to a definite sequence of events which transpire at the junction of the shaft of the bone (diaphysis) with its head (epiphysis). The shaft is composed of growing bone, the epiphysis of cartilage which is largely in a resting state. As the animal grows the cartilage of the epiphysis is converted into bone through (a) encroachment on it of the bony shaft and (b) through the expansion of one or more centers of ossification which form in it. The process of growth in length of the shaft is the same as results in the expansion of the centers of ossification in the epiphysis.

The cells of the resting cartilage have no particular arrangement until they come within a short distance of the shaft or center of ossification. The cartilage cells then arrange themselves into columns parallel to the long axis of the shaft or the radii of the ossification center. This columnar zone is known as the "proliferative cartilage." Lime salts are deposited in the matrix separating the cells of the thin layer of cartilage in contact with the shaft. This is known as the zone of provisional calcification. Cartilage cells in this zone lie in a matrix which is like a calcified honey-comb. As the shaft grows tiny blood vessels sprout evenly from it and each vessel opens a single compartment in the calcified honeycomb. The cartilage cell is freed to degenerate or to become an osteoblast or bone-forming cell. Large numbers of osteoblasts follow the blood vessel sprouts into

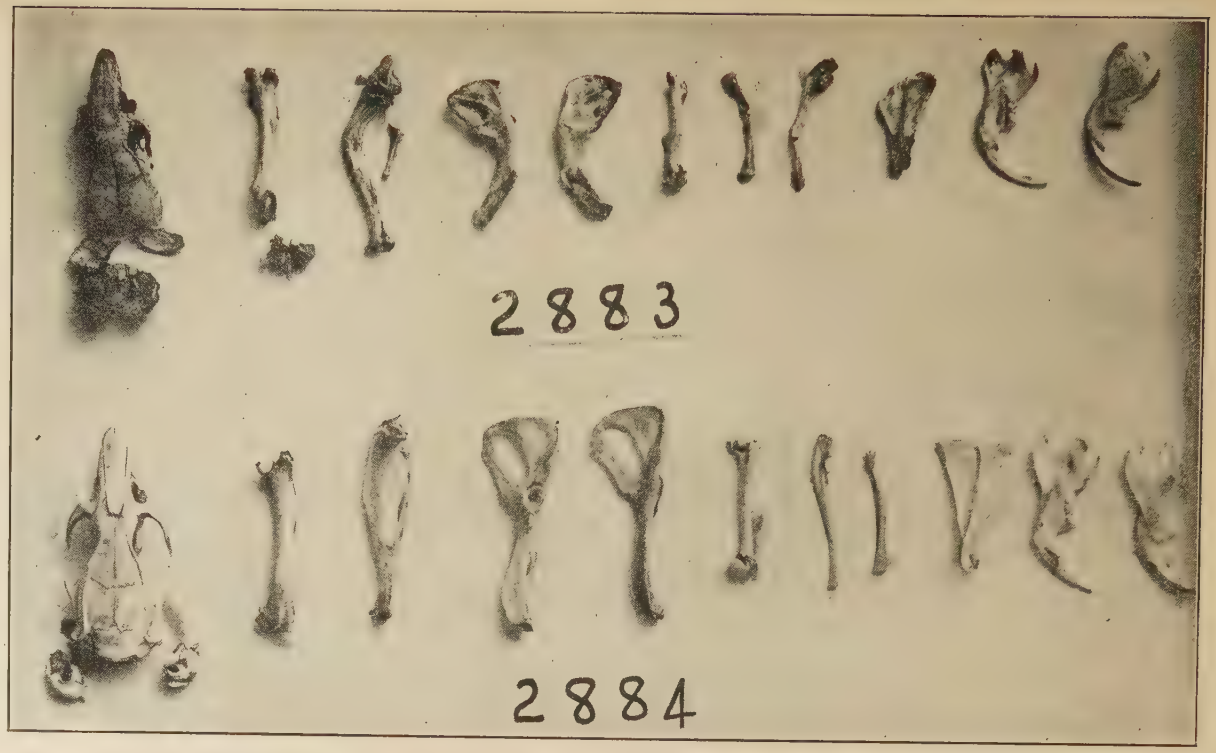


FIG. 18.—Illustrates the marked difference in the quality of the skeleton of rats which were fed on diets which were poor and good respectively. The bones at the top (Lot 2883) are those of an animal whose diet was satisfactory except that it was too poor in calcium. Note the dark coloration due to hemorrhage; the detachment of the head from the femur; the separation of the bones of the skull; the deformity of the pelvic bones; the crooked form of the radius and ulna; the inferior development of the scapula and the failure of the bone of the mandible to grow out around the base of the incisors as far as it should. Note also that these incisors are growing at wrong angle and are too long and poorly formed.

Rat 2884 took the same diet as the above but with calcium carbonate added. Its bones were much lighter in color, and were dense and well formed.

the cartilage. These settle on the spicules of calcified matrix which remain after the cartilage cell capsules are opened. These spicules furnish cores about which the trabeculae of bone in the shaft are built. The osteoblasts on the spicules of calcified matrix bury themselves in a tissue known as osteoid which they manufacture. This receives a deposit of lime salts and becomes bone.

Growth in thickness of the shaft is accomplished by cells in a thick layer of connective tissue which surrounds the shaft and is called the periosteum. These connective-tissue cells are transformed directly into osteoblasts and lay down bone in layers beneath the periosteum. During growth, and to a lesser extent after growth has ceased, the structure of a bone is subject to constant change to meet the requirements of exercise and changing conditions of stress and strain. Old bone is constantly being removed (resorption) and new bone is always being formed. In a normal, healthy bone these processes are always in a dynamic equilibrium.

Abnormal Histological Changes in Rachitic Bones.—In the rachitic bone all these processes of growth and the maintenance of equilibrium are abnormal. Calcium salts are not deposited in a normal way. In a normal bone the junction of the cartilage with the shaft forms a straight, regular line. In the rachitic bone it is ragged because there is no provisional zone of calcification and the cartilage is invaded by large tufts of blood-vessels from the shaft which destroy it irregularly. At the same time masses of cartilage persist where they should be destroyed and islands remain cut off from the main body of the tissue. Osteoid is not calcified as it is formed either in the trabeculae or by the periosteum, so that the bones become soft. The animal attempts unsuccessfully to compensate for the weakness due to the lack of lime salts by producing osteoid tissue in abnormal amounts, especially at points subject to stress and strain (the curved side of the bone and at the insertions of muscle and tendons). This overproduction of osteoid and cartilage causes irregular enlargement of the bones and their weakness consequent upon the lack of lime salts results in bowing and fracture. Because of the abnormal growth at the end of the shaft a zone known as the rachitic metaphysis forms, which consists of blood-vessels, connective tissue, osteoid, marrow elements and cartilage cells in all stages of degeneration and change into other tissue. The osteoid formed shows little sign of being absorbed in rickets as it is com-

monly seen. The balance of forces has swung in the direction of apposition and persistence of cartilege. In other words, a typical rachitic bone has the following characteristics: (1) there is no calcification of the provisional zone of cartilage; (2) cartilege cells persist in the shaft below the normal epiphyseal line; (3) there is an irregular invasion of the cartilage by blood vessels and connective tissue; (4) newly formed preosseous tissue remains uncalcified and is produced in abnormal amounts—the so-called osteoid tissue; (5) a rachitic metaphysis which is composed of blood vessels, isolated masses and tongues of cartilage cells, connective tissue and osteoid is formed.

Importance of Phosphorus in Study of Rickets.—One of the diets which we have had most experience with produced rickets (2677) but it also produced xerophthalmia. We were able to produce rickets without xerophthalmia, but we had not been able to do the reverse. Diet 2677 was composed of rolled oats, flaxseed oil meal, sodium chlorid, calcium carbonate and dextrin. We had used up to this time only sodium chlorid and calcium carbonate with this diet and so had produced rickets. We now tried the addition of our 185 salt mixture to this ophthalmia-producing diet in order to see whether we could produce ophthalmia without rickets. The rats developed ophthalmia but did not develop rickets. This suggested the relation between the calcium and phosphorus content of the diet as influencing bone growth.

Phemister (1918) confirmed the observations of Wegner (1872) who fed 1/400th of a grain of yellow phosphorus daily to growing dogs, cats and chickens over considerable periods of time, and observed definite changes in the long bones. There was new bone formation in the diaphysis which was especially marked along the epiphyseal lines, leading to the formation of a dense layer in the end of the shaft which varied in thickness with the length of the period of phosphorus administration. Phemister showed in roentgen-ray studies the influence of phosphorus on the growth of normal bones and the bones in the growth disturbance of osteogenesis imperfecta, and dyschondroplasia. He later found (1921) that the end-results produced by phosphorus, and the combination of phosphorus and cod liver oil were quite similar. Rarefied areas of the rachitic bones acquired an approximately normal density in both instances.

Sherman and Pappenheimer (March, 1921) published an important paper demonstrating that rickets could be produced in

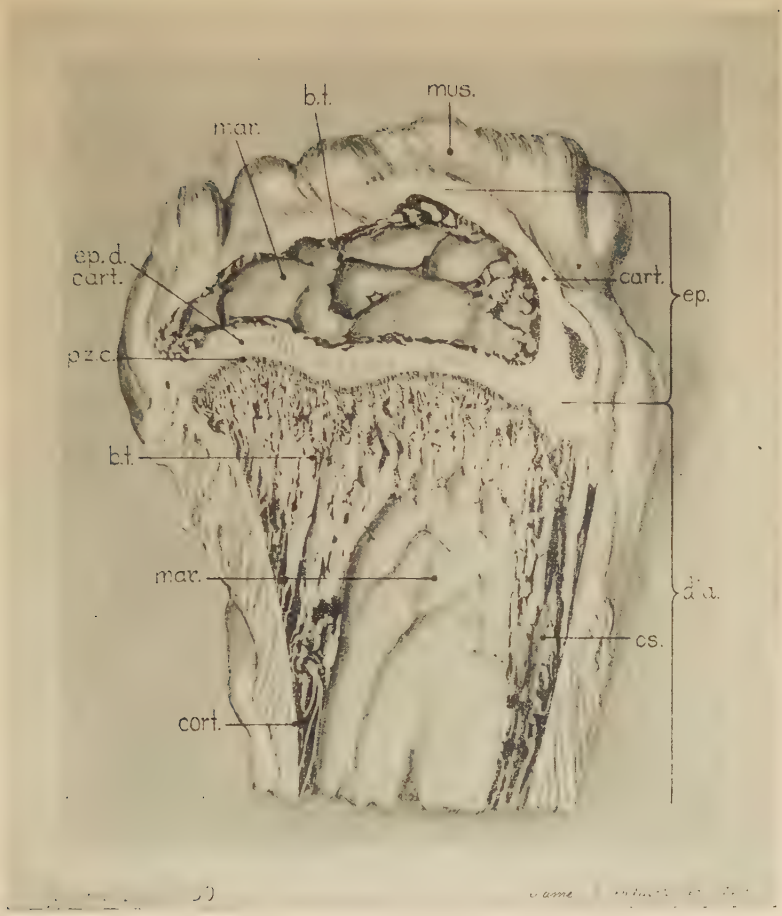


FIG. 19.—Normal bone of a rat (proximal end of tibia). This is to be contrasted with Fig. 20, which is a rachitic bone, and also with Fig. 21, which shows advanced healing. (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. Johnson, Jr., of the Mead Johnson Biological Laboratory.)

Legend: b.t.—bone trabeculae; cart.—cartilage; cort.—cortex; dia.—diaphysis; ep.—epiphysis; ep.d. cart.—epiphyseal diaphyseal cartilage; mar.—marrow; mus.—muscle; p.z.c.—proliferative zone of cartilage; os.—osteoid; met.—metaphysis.

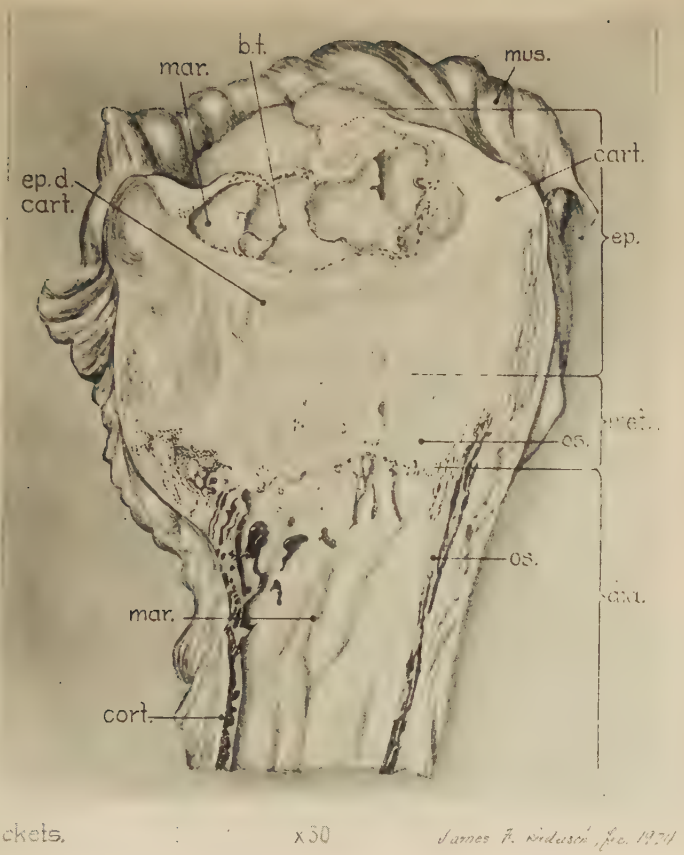


FIG. 20.—Bone of a rachitic rat (proximal end of tibia). This bone shows no healing on the diaphyseal side of the metaphysis. This shows the florid rickets produced by diet 3143. Note the enormous band of cartilage cells, the wide metaphysis which is absent from the normal bone, and the excessive amount of osteoid tissue. (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. Johnson, Jr., of the Mead Johnson Biological Laboratory.)

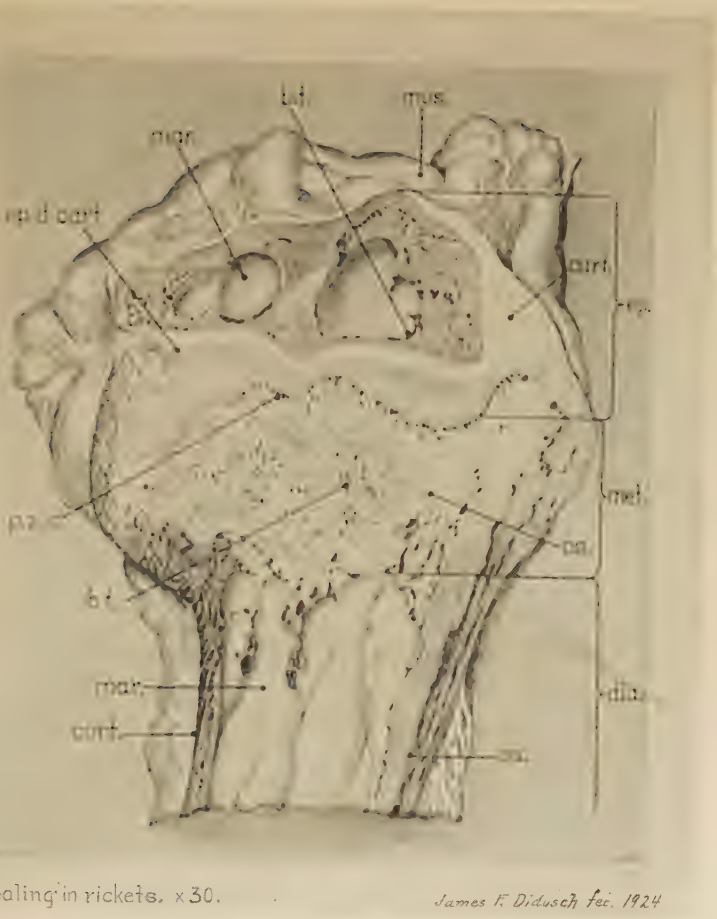


FIG. 21.—First stage of healing of a rachitic bone. When cod liver oil is administered to an animal whose bone is like this shown in Fig. [illegible] calcification takes place as shown at [illegible] (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. Jenkins, Jr., of the Mead Johnson Biological Laboratory.)

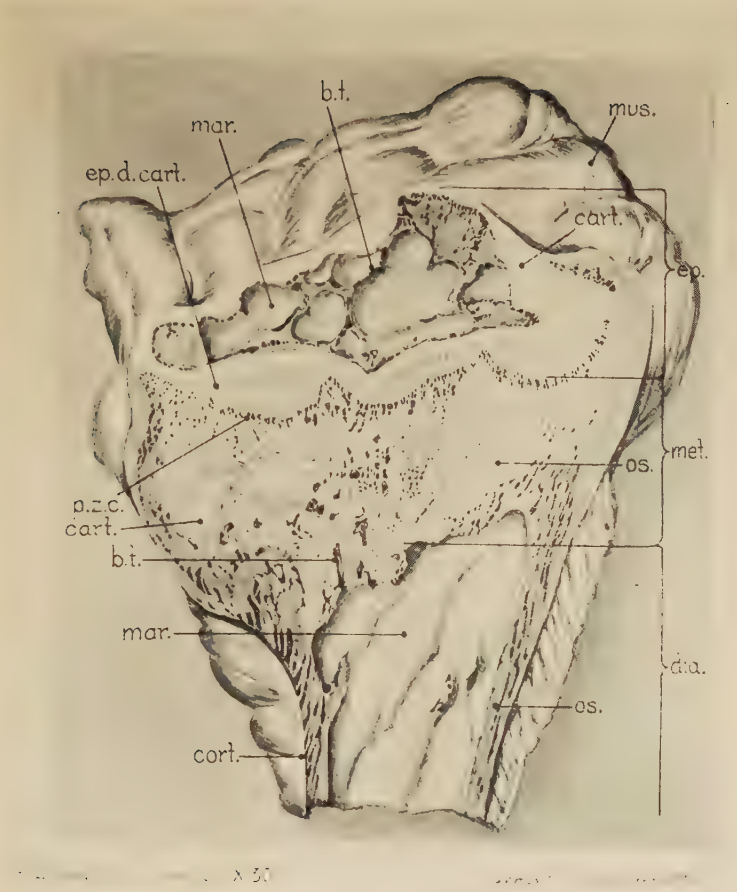


FIG. 22.—Later stage of healing of a rachitic bone than that shown in Fig. 21. The calcification is shown in black at p.z.c. When the administration of cod liver oil is continued the bone returns toward the normal structure shown in Fig. 19. If the disease is severe the bone calcifies with deformities. (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. Johnson, Jr., of the Mead Johnson Biological Laboratory.)

rats on a diet low in phosphorus and that it could be prevented by the addition of alkaline potassium phosphate. Their diet was composed of patent flour 95, calcium lactate 3, sodium chlorid 2, with or without the addition of ferric citrate. This diet regularly produced rickets which possessed the characteristics of the disease in the human being. When alkaline potassium phosphate was added to the diet in the proportion of 0.4 per cent and an equivalent amount of calcium lactate withdrawn, the animals were completely protected against rickets. This observation clearly demonstrated that rickets can be prevented by the addition of the phosphate ion under certain dietary restrictions.

Shipley and co-workers (May, 1921) reported similar experiments. They reported the production of rickets by means of two diets. The diet of Lot 2667 was composed of rolled oats, flaxseed oil meal, sodium chlorid, calcium carbonate and dextrin. The diet of Lot 2806 contained rolled oats, gelatin, sodium chlorid, calcium carbonate and dextrin. Both diets were deficient in vitamin A and phosphorus. When a complete salt mixture containing potassium phosphate replaced the sodium chlorid and calcium carbonate the diets no longer produced rickets but typical osteoporosis. The observation of Sherman and Pappenheimer and of Shipley and co-workers marked a distinct advance in the investigation of rickets. They showed the importance of the inorganic constituents of the diet, especially of phosphorus in relation to the disease. With the exception of Mellanby, who had placed but little importance on the salt composition of the diet, almost all other investigators have sought to produce rickets by a reduction in the calcium. Pappenheimer and co-workers (1921) confirmed the observation that the protection against rickets in rats which was conferred by basic potassium phosphate was conferred by the phosphorus and not the potassium. They presented data which showed that in rats the absence of vitamin A does not produce rickets nor does its presence prevent it.

Low Calcium Rickets.—McCollum and co-workers (1921) studied the effect on growing rats of diets deficient in calcium. The phosphorus content was about optimal (0.41) and 3-5 per cent of butter fat supplied vitamin A. The bones of these rats differed in certain instances from those of rats on diets deficient in phosphorus. The metaphysis was narrow. The osteoid tissue about the trabeculae was much more ragged in these rats than in those fed diets deficient in phosphorus. Many evidences of

resorption of the trabeculae were present. Bones which showed evidences of resorption invariably contained numbers of large cells, the cytoplasm of which was filled with large basophile granules. There were certain changes in these bones which differentiated them from the rickets seen in children. The cartilage cells retained more of their columnar arrangement in spite of a considerable invasion by blood vessels and the evidences of bone resorption in the diaphysis were much more extensive than that seen in the high calcium, low phosphorus rickets. Shipley and co-workers (1921) stated "whatever the defects in the salt composition in the diets employed, the effect of the addition of cod liver oil was greatly to improve the growth and condition of the animal as a whole, and to shift the pathological condition in the bones toward the normal condition." As a result of their observations they believed it probable that the function of the organic factor in cod liver oil was to activate and co-ordinate cellular function. The efficiency of the organism was apparently raised and could put into successful operation defense mechanisms without which it would have been ineffectual. They believed that cod liver oil did not regulate the inorganic metabolism but permitted the body's own regulatory mechanisms to operate with increased efficiency and economy.

Low Phosphorus—High Calcium Rickets.—Shipley and co-workers (1921) reported the production of rickets by means of a diet (Lot 3127) relatively high in calcium, very low in vitamin A and in phosphorus. The calcium was high only in the sense that it was excessive in proportion to the phosphorus content which was extremely low. The phosphorus content was 0.209 gm. and the calcium about 0.8 gm. This diet produced both rickets and xerophthalmia in little rats confined to it for 5 to 6 weeks. When 0.5 per cent of butter fat was added to this diet (Lot 3133) the animals lived somewhat longer and the pathological condition was more intensified, probably due to the slight stimulation given by the butter fat to the growth of bone.

When the level of phosphorus in diet 3127 is raised so that both calcium and phosphorus are present in presumably adequate amounts so that the deficiency is limited to a lack of vitamins A and D, rickets does not develop. A deficiency in vitamin D alone cannot produce rickets. When no calcium carbonate is added to this diet so that the quantities of calcium and phosphorus are both low, rickets does not develop. When the calcium carbonate of diet 3127 was replaced by 2 per cent of calcium

TABLE XIX

Composition of Diets (Percentage Composition)

No. of Diet	Wheat	Maize	Polished Rice	Rolled Oats	Peas	Navy Beans	Gelatin	Wheat Gluten	NaCl	$CaCO_3$	KCl	Flaxseed Oil Meal	Butter Fat	Dextrin	Salts 185
2667				40					1.0	1.5		8.3		49.2	
2806				40			10		1.0	1.5				47.5	
3120				40			10							46.3	3.7
2638	30	30	10	10	10	10									
3127				40			10	7	1.0	2.0	1.0			39.0	
3133				40			10	7	1.0	2.0	1.0		0.5	38.5	
3143	33	33					15	15	1.0	3.0					

phosphate so that the diet was deficient in the organic factor alone, the pathological condition which developed in the skeleton was not rickets but osteoporosis. It was such observations that led us to an appreciation of the significance of the quantitative relationships between calcium and phosphorus in the diet.

Cod Liver Oil Versus Butter Fat for Bone Growth.—Shipley and co-workers (1921) discussed the relative effectiveness of cod liver oil as contrasted with butter fat for protecting the rat against an insufficiency of calcium in the presence of what under normal conditions is about a normal phosphorus supply. They pointed out that with a diet containing seeds plus casein 10, a diet low in calcium, 1 per cent of cod liver oil was very superior to even 20 per cent of butter fat in inducing growth and well-being in the animals. They were not willing at that time to make the statement that a separate factor distinct from vitamin A was to be found in cod liver oil. They stated: "We have not found it possible to demonstrate experimentally a difference between the effects of cod liver oil and butter fat when the content of the diet in calcium and phosphorus is near the optimum, the other elements in the diet being satisfactory." They further stated: "These results suggest the possibility that a dietary essential distinct from the anti-ophthalmia substance (fat-soluble A) may exist. If this is the case, this would appear to be present in butter fat in small amounts but to be very abundant in cod liver oil." Hess and co-workers (1921) attempted to produce rickets in rats on a diet deficient only in vitamin A. The rats did not develop rickets but did develop xerophthalmia. These investigators used a complete salt mixture. They apparently were not aware that rickets could not be produced in animals fed a diet in which the calcium and phosphorus contents were near the optimal.

Line Test Diet 3143.—Our experience had shown that a diet of cereals and legume seeds plus casein 10, plus calcium carbonate 3 per cent, induced rickets in animals. We fed another group of animals the diet but increased the casein to 20 per cent, leaving the calcium carbonate at 3 per cent. These animals grew well and had normal bones. We had at this time made the observation, as had also Sherman and Pappenheimer, that the phosphate ion under certain conditions would protect against rickets. Since casein is a phosphorus-containing protein, we decided to replace it in this diet by the non-phosphorized proteins gelatin and wheat gluten, leaving 3 per cent of calcium

carbonate in the diet as before. Diet 3143 has the following composition: Wheat* 33.0, maize 33.0, gelatin 15.0, wheat gluten 15.0, sodium chloride 1.0, and calcium carbonate 3.0 per cent. Since the diet contained considerable amounts of vitamins A and B, and proteins of good quality, the animals grew and remained in a fairly good state of nutrition for varying periods depending on the season of the year. After about 40 days on this diet (Lot 3143) we noted that the animals did not have full control of the hind legs. They had a peculiar gait due to a partial loss of control of the posterior extremities. The gait was tottering and the hindquarters wavered slightly from side to side. When the animal started to move off rapidly it hopped, usually favoring one hind leg. This diet always produces a florid condition of rickets, and calcification of the cartilage is wanting. This diet produces a much more exaggerated picture of rickets than that seen in human beings. If this diet is fed with 2 per cent of calcium carbonate instead of 3 per cent the histo-pathological picture is identical with that seen in the rickets of children. Diet 3143 differs from diets 3127 and 3133 in having a higher calcium to phosphorus ratio than either. Diet 3143 induces much more growth in the animals than either of the latter two diets.

Diet 3143 produces the following changes in the bones of rats: The bones are very soft. The ends are very much enlarged. The enlargement of the ends of the long bones is due to an increased depth and probably also to an increased breadth in the epiphyseal cartilage, and to the presence of an enormous metaphysis. The cartilage in every animal is entirely free from lime salt deposits. Some irregular lime salt deposition is sometimes present in the metaphysis of certain animals. The cortex is thin. It is composed of bone with scanty central cores of calcified material with broad osteoid borders. The breadth of the osteoid borders is extreme in these animals. Few osteoblasts, or at least cells which could be identified as such, could be found lining the osteoid trabeculae. The evidences of resorption of the calcified portions of the trabeculae were slight. It will be recalled that in those animals suffering from low calcium, high phosphorus rickets, a considerable degree of resorption was taking place.

As has been pointed out, a rachitic bone does not form a provisional zone of calcification, since the animal fails to deposit lime salts in the inter-cellular substance of the cartilage. The

* Hard wheats are generally much richer in phosphorus than soft wheats. Soft wheat should be employed in making this diet.

proliferative cartilage persists in some places and in others is destroyed in an irregular way by aberrant capillaries which sprout in a disorderly fashion from the parent vessels in the shaft and invade the cartilage in all directions. The line of junction of the epiphysis and shaft becomes very irregular and long tongues and islands of cartilage cells are left isolated in the metaphysis and diaphysis. At the same time the bone is not formed normally either as regards the direction of the trabeculae, since there is no calcified intercellular substance to serve as a guide for the activity of the osteoblasts, or as regards calcification, since calcium salts are not deposited in the osteoid tissue. Osteoid is formed in excessive amounts, perhaps as an attempt at compensation for the loss of strength of the bones from failure of the pre-osseous tissue to calcify.

Animals which show this wide metaphysis free from calcification are ready to be used for determining the presence or absence of vitamin D in food-stuffs. They must at this point be kept in individual cages in order that the food consumed can be recorded. McCollum and co-workers (1922) demonstrated that animals in this condition will produce a positive "line test" if allowed to starve. Howland and Kramer (1922) showed this calcification to be accompanied by changes in the calcium and phosphorus in the serum. They found serum of rats on diet 3143 to contain about 10.5 mg. of calcium and about 3 mgm. of phosphorus per 100 c.c. After a few days' starvation the calcium was markedly lowered, whereas the phosphorus may be increased to 16 mgm. per 100 c.c. of serum. This produces the healing seen in starvation of these rachitic rats. In testing animals for the anti-rachitic properties of food-stuffs it has been our custom to examine the distal end of the left femur and the proximal end of the left tibia for reformation of the provisional zone of calcification. The bones are split in two with a sharp scalpel and one-half of each is fixed in 10 per cent formaldehyde, decalcified in Müller's fluid and imbedded in parloidion. The other half of each bone is immersed in 1 per cent of silver nitrate and exposed to the sunlight or to the light of an arc light and studied through a binocular microscope.

Rats which give a positive "line test" differ from the controls in having a broad linear deposit of calcium salts on the metaphyseal side of the epiphyseal cartilage. The band, which may not be complete, is separated from the shaft of the bone by the depth of the metaphysis and from the nucleus of ossification of

the epiphysis by the depth of the epiphyseal cartilage. It can be seen on a freshly cut surface of an untreated bone as a yellow line which marks the epiphyseal border of the metaphysis. The deposit is blackened by 1 per cent silver nitrate in fresh gross specimens. It appears like a cross section of a black honeycomb when it is examined with a binocular microscope. The metaphysis of these bones usually appears to be congested. The calcium salt deposit is in the proliferative zone of the cartilage. It may extend completely across the bone or may be interrupted or fragmentary according to the activity of the calcium-depositing substance. It is stained brown in permanent sections by silver nitrate, or an intense blue by hematoxylin.

Is Rickets in Animals the Same Disease as in Humans?—Findlay (1922) questioned whether the rickets induced in animals is the same disease as the rickets of human beings. Park (1923) regards the rickets produced in rats to be identical with that seen in human beings; the pathological conditions found to exist in the blood are identical; the rickets produced in animals may or may not be accompanied by the symptoms of tetany exactly as human rickets may or may not be accompanied by tetany; the same remedial measures are effective in both. To quote: "Not the slightest doubt can exist that the rickets produced experimentally in animals and the rickets occurring in human beings is the same disease."

Pappenheimer (1922) investigated the nature of the curative principle contained in cod liver oil. He described the changes which took place in the bones in rickets after its administration. He found that the healing of rickets depends not only on the length of time that the rat is under treatment but also depends on the activity of the cod liver oil used and on the dosage. He summarized the evidences of healing found as follows: (1) a deposition of calcium in the distal half of the widened zone of preparatory calcification; (2) invasion of the calcified portion from the lateral and subchondral sides by large blood vessels, with destruction of the cartilage cells; (3) changes in the osteoid tissue which may be taken to indicate beginning lysis or halisteresis; (4) great dilation or congestion of the blood sinuses in the metaphyseal region; and (5) deposition of granular calcium in the perichondral and pericortical osteoid.

Low Calcium Rickets—Low Phosphorous Rickets.—Shipley and co-workers (1922) found that a pathological condition corresponding in all fundamental respects to rickets in human

beings can be produced by diet in two ways: (1) by diminishing the phosphorus and supplying an optimal amount or an excess of calcium; (2) by reducing the calcium and maintaining the phosphorus at a concentration somewhere near the optimum. As a result of these experiments they were led to believe that there are two kinds of rickets, one being characterized by a normal or nearly normal blood calcium and a low blood phosphorus (low phosphorus rickets; calcium: phosphorus ratio large), the other by a normal or nearly normal blood phosphorus but a low blood calcium (low calcium rickets; low calcium:phosphorus ratio). The investigations of Howland and Kramer (1921) and of Kramer, Tisdall and Howland (1921) on the calcium and phosphorus content of the blood serum in rickets and tetany supported this view. These observers found that in children suffering from rickets alone the phosphorus of the blood serum is low and the calcium about normal; in children suffering from tetany complicating rickets, on the other hand, the calcium is low but the phosphorus not far from normal. They believe that tetany is an expression of the nervous tissues of an insufficiency of the calcium ion, and that rickets is essentially an expression on the part of the skeleton of disturbed relations between the calcium and phosphate ions of the body fluids. They stated that tetany is frequently associated with rickets because rickets is a disease in which the calcium ions in the body tissues and fluids are subject to variations. Tetany occurs independently of rickets just as rickets occurs independently of tetany. Since tetany may occur with a low phosphorus form of rickets it does not serve to mark off one form of rickets from the other. Tetany is essentially associated with a low calcium form of rickets and for all practical purposes the low calcium form of rickets is the rickets of tetany.

Pappenheimer and co-workers (1922) confirmed the observation that rachitic bone lesions can be produced in rats by a diet containing an excess of calcium but deficient in phosphorus or vice versa. They further pointed out that inorganic salts other than calcium or phosphorus seem to be without influence upon the development or prevention of rachitic lesions. They found that the phosphorus in casein does not completely prevent the development of rickets when substituted for an equivalent amount of basic K_2HPO_4. They found the protection given by lecithin to be equal to its phosphorus content; that given by yeast was at least proportional to its phosphorus content. The sub-

stitution of 10 per cent of egg albumin improved the nutrition but did not prevent rickets. The addition of meat to the low phosphorus diet induced normal growth and normal bone formation because of its phosphorus content. Hess and co-workers (1922) observed the spontaneous healing of rickets in rats. They believe it to be due to the animals remaining at a stationary weight which lowered the phosphorus requirement and caused a rise in the phosphorus content of the blood. Gutman and Franz (1921-22) reported the production of rickets in rats fed diets containing various amounts of calcium and phosphorus. They found the blood phosphate to be reduced parallel to the degree of severity of the rachitic lesion.

Demonstration of Existence of Vitamin D.—Although in January, 1922, McCollum and co-workers presented evidence which was all but conclusive that there is in cod liver oil and butter fat a calcium depositing substance distinct from vitamin A, it could not be looked upon as finally proven. They stated: "The results of this series of experiments were so consistent that we can deduce no other conclusion than that cod liver oil contains in abundance some substance which is present in butter fat in but very slight amounts and which exerts a directive influence on bone development and enables animals to develop with an inadequate supply much better than they could otherwise do. This substance is apparently distinct from fat-soluble A, which is essential for growth and which is associated definitely with the prevention of ophthalmia (keratomalacia)." Our experience with diets very low in calcium but containing cod liver oil or butter fat demonstrated that 1 per cent of cod liver oil was very superior to 20 per cent of butter fat with these diets. The possibility remained that butter fat and cod liver oil contained one and the same substance (vitamin A) but that cod liver oil contained much more of this. Zilva and Miura (1921) stated their belief that cod liver oil was 250 times as potent as butter fat as a source of vitamin A.

Our data demonstrated that coconut oil did not prevent sore eyes but with certain low calcium diets it induced better growth in animals than did olive oil. There were suggestions in Mellanby's paper that some of his dogs fed coconut oil were in better condition than those fed olive oil. His data were not sufficiently clear cut to establish anything definite concerning the reason for this.

Our experience had convinced us that existing methods were

incapable of differentiating beyond doubt between vitamin A and a special calcium-depositing substance, should this exist. McCollum and co-workers (1922) formulated a plan which involved a comparison of a selected list of fats in respect to three kinds of effects in nutrition: (1) we tested cod liver oil, shark liver oil, butter fat and several vegetable oils for their potency in causing the cure of xerophthalmia due to the lack of vitamin A; (2) we made comparative tests of the same fats to determine their value in promoting growth in young rats which were restricted to a diet so low in calcium that satisfactory growth was not possible without the provision of some substance which would make for greater efficiency in the utilization of calcium than that which could be effected in its absence; (3) we further studied these same fats by means of our "line test" to discover their relative values for inducing the deposition of the line of calcium salts in rachitic bones. With the data secured from these three distinct types of tests we were able to interpret accurately the results of much of the experimental data in the literature which were confusing.

EXPERIMENTAL PROCEDURE

I. Test for vitamin A: The diet used for testing the value of different fats for the cure of xerophthalmia had the following formula:

Lot 3392

	per cent
Rolled oats	40.0
Casein	5.0
NaCl	1.0
$CaCO_3$	1.5
Dextrin	52.5

This diet is essentially lacking in vitamin A. If 2 per cent of butter fat or cod liver oil replaces an equivalent amount of dextrin xerophthalmia never develops. In making the test referred to young rats of about 40 gm. in weight were restricted to this diet until the puffiness of the eyelids was distinctly evident. On the addition of sufficient fat containing vitamin A at the time when the edema of the eyelids is becoming severe, the swelling rapidly disappears and the eyes return to a normal appearance within a few days. Two per cent of cod liver oil, 3 per cent of shark liver oil, 3 per cent of burbot liver oil or 2 per cent of butter fat were found to effect a prompt cure of incipient xerophthalmia under the conditions of our test. Although we

had data from other experiments which indicated that certain of the vegetable oils when fed liberally (8-20 per cent) from the beginning of the experiment tended to defer the onset of xerophthalmia, we have found that such amounts of vegetable fats would not cure the eye condition after it had once developed. It should be especially noted that 15 per cent of coconut oil did not cure or prevent xerophthalmia.

Hopkins (1920) was the first to point out that oxidation destroys vitamin A. He showed that if oxygen was allowed to pass through heated butter fat the vitamin A was readily destroyed. With this treatment butter fat lost its power to induce growth or to cure ophthalmia of dietary origin. Mellanby attempted (1921) to make use of this means of destroying vitamin A in order to determine whether there is a distinct "anti-rachitic substance." He found butter fat of little value for protecting against rickets after it had been oxidized, whereas cod liver oil after the same treatment, *i.e.*, heated to 120° C for 4 hours while oxygen was passing through it, still protected his animals against rickets. He stated: "If it should happen that 4 hours' heating and oxidation at 120° C also leaves a large amount of fat-soluble A in cod liver oil, it will go a long way, especially when considered together with the butter results, to clinch completely the identity of fat-soluble A and the anti-rachitic vitamin." Mellanby used no method of testing for vitamin A as distinct from the calcium depositing substance since he did not make use of the ophthalmia test for vitamin A.

We found that cod liver oil treated with a stream of air bubbles at the temperature of boiling water for 12 or 20 hours no longer contains sufficient vitamin A to relieve rats from xerophthalmia when administered to the extent of 2 per cent of the diet. Untreated cod liver oil under these conditions invariably causes complete recovery within 5 days. Likewise, 2 per cent of fresh butter fat under exactly comparable experimental condition effects the disappearance of opthalmia within 5 to 10 days. The significance of these results in connection with the problem of the existence of a special calcium-depositing vitamin will be discussed later.

II. Tests of fats for their protective power against the effects of deficient calcium supply. Shipley and co-workers (1921) had described experiments which showed clearly that a diet may be so deficient in calcium as to prevent growth in young rats and that butter fat failed to protect the animals from the effects

of calcium deficiency, whereas the same diet supplemented with cod liver oil may promote good growth. We modified the technic used in these earlier experiments to some extent for studying the influence of fats on calcium metabolism. For this purpose we used the following diet:

Lot 2947

	per cent
Whole wheat	25.0
Whole maize	19.5
Polished rice	9.5
Rolled oats	9.5
Casein	10.0
Whole milk powder	5.0
Peas	9.5
Navy beans	9.5
NaCl	1.0
Dextrin	1.5

The dietary properties of this food mixture had been carefully determined. The proteins are of good quality and are abundant (about 23 per cent). Its content of vitamin B is entirely adequate. It is somewhat deficient in vitamin A, but not sufficiently so to induce xerophthalmia even if animals are confined to it for a long period. Its inorganic content is such as to induce skeletal deformities. The diet is low in calcium, but not far from the optimal in phosphorus (if calcium had been adequate). Our problem was to see to what extent, if any, the different fats increased the efficiency of the animals in utilizing the small amount of calcium at their disposal.

III. Tests of fats for their power to stimulate healing in rickets. The foregoing data relative to the values of several fats for relieving ophthalmia and for increasing the efficiency of the tissues in utilizing calcium when this element is present in very inadequate amounts in the diet, could not be looked upon as a safe basis for deciding whether the effects in both types of experiments were due to vitamin A or to two distinct substances. The results of the experiments in which coconut oil was studied strongly suggested that this oil contained a substance which improves the utilization. It had also been pointed out that on the basis of experimental tests coconut oil does not contain the substance vitamin A which relieves ophthalmia. We, therefore, supplemented the data obtained by the above described tests with observations on the effects of the several fats on the initiation of the healing process in rickets. For this purpose we used

the technic which we have called the "line test." Diet 3143 has the following composition:

	per cent
Whole wheat	33.0
Whole maize	33.0
Gelatin	15.0
Wheat gluten	15.0
NaCl	1.0
$CaCO_3$	3.0

This diet contains an inadequate amount of vitamin A but sufficient to prevent xerophthalmia. Although the diet contains an excessive amount of calcium no deposition of calcium salts takes place in the growing bones. A discussion of the effects produced in bones by this diet has been given on page 398. After the animals had been confined to this diet for about 35 days the various oils were administered. We included maize oil and sesame oil among the vegetable oils examined.

Coconut oil was found to be distinctly more effective as a calcium depositing agent than any of the other vegetable oils tested. It was, however, inferior to butter fat or any of the fish oils tested. The oils studied are listed in the following table. The amounts of the oils fed, the number of days of administration, the length of the preliminary period, the number of animals tested, and the results of the tests, are given. Since starvation causes the deposition of calcium salts in the bones under the conditions of the test, we kept accurate records of food consumption in making these tests.

An inspection of the table shows that cod liver oil, shark liver oil and burbot liver oil were highly effective in moderate doses in causing the deposition of calcium in the bones of rachitic animals. Butter fat was also effective when fed in large amounts (15 to 30 per cent) but it was necessary to extend the time of administration to 14 days in order to obtain even a faint calcification of the bones. Coconut oil when fed at 20 per cent of the diet for 15 days caused the deposition of small amounts of calcium salts in the bones under the conditions of our test. Maize oil, olive oil, cotton-seed oil and sesame oil were likewise fed at 20 per cent of the diet for 14-15 days but in no case was there any tendency to the deposition of calcium salts. Samples of cod liver oil which had been oxidized for 4, 12 and 20 hours respectively were tested for their calcium-depositing properties. Those oxidized for 12 and 20 hours showed this potency to a degree apparently comparable with similar amounts of un-

TABLE XX

Kind of oil.	*Per cent.*	*Number of days in preparatory period.*	*Number of days fat was given.*	*Number of animals.*	*Results.*
Cod liver oil.	2	21	5	6	Healing rickets.
" " "	2	25	6	2	" "
" " "	2	28	11	3	" "
" " "	2	49	5	3	" "
" " "	2	49	5	3	" "
" " "	0.2	70	5	1	Severe rickets, a few specks of calcium in cartilage.
" " "	0.4	70	5	1	Severe rickets, no healing.
" " "	0.6	70	5	1	Beginning healing of rickets.
Cod liver oil (oxidized 4 hrs.)	2	25	6	3	Healing rickets.
Cod liver oil (oxidized 12 hrs.)	2	28	11	3	" "
Cod liver oil (oxidized 20 hrs.)	2	29	10	3	" "
Butter fat.	30	21	14	2	Beginning healing.
" "	30	32	11	4	" "
" "	15	17*	12	4	3 of these animals showed occasional specks of calcium in cartilage.
" "	15	20	20	1†	Beginning healing rickets.
Shark liver oil.	2	34	11	3	Marked healing.
Burbot liver oil.	2	30	6	2	Healing well on.
" " "	2	30	10	4	" " "
Coconut oil.	20	24	15	5	Very slight evidences of healing.
Maize oil.	20	34	15	5	Severe rickets, no healing.
Olive "	20	34	15	5	" " " "
Cottonseed oil.	20	29	14	5	" " " "
Sesame oil.	20	24	15	5	" " " "

* This preparatory period was too short. The metaphyses were narrow and irregular. It was possible to mistake calcified matrix which had existed before the onset of the disease as evidence of healing.

† This animal was badly deformed and feeble after being fed the butter fat for 20 days. There was no clinical evidence that the butter fat had been at all beneficial.

oxidized samples, notwithstanding the fact that they had entirely lost their power to cure xerophthalmia. The samples which had been oxidized for 4 hours still cured xerophthalmia when 2 per cent of the diet consisted of the oil. They likewise gave a positive test for the calcium-depositing substance.

Our results are in harmony with those of Mellanby in that they show that coconut oil has an anti-rachitic effect. They prove conclusively that this effect is not due to the vitamin A in the fat. Many of Mellanby's apparently discordant results we believe can be accounted for on the basis of his not appreciating the significance of the importance of the ratio between the calcium and phosphorus in his diets, rather than on the content of his diets in the calcium-depositing vitamin.

The evidence which we set forth in this paper demonstrated that the power of certain fats to initiate the healing of rickets depends on the presence in them of a substance which is distinct from vitamin A. These experiments demonstrate clearly the existence of a vitamin whose specific property so far as we can tell at present is to regulate the mineral metabolism of the bones.

Availability of Calcium Salts.—McCollum and co-workers (1922) compared calcium lactate, chlorid and carbonate in the production of rickets when fed at levels to introduce the same amounts of calcium, using their "line test" diet 3143. We found that the chlorid and carbonate produced rickets in an exaggerated form. The lactate produced rickets of the type commonly seen in children. It resembled the rickets which we have produced with diet 3143, but using 2 per cent of calcium carbonate instead of 3 per cent. There were abortive attempts at healing with reformation of the provisional zone of calcification, which left traces in the form of more or less complete lines of calcified inter-cellular substance in the cartilage and metaphysis of the bones. We could not detect any influence of the chlorid, which contains a non-oxidizable radical, in contrast with the carbonate or lactate, which are both potential neutralizers of acid in the body. We also discussed the conditions which must be fulfilled in preparing animals for testing the anti-rachitic properties of individual food-stuffs. Steenbock and co-workers (1923) in studying the availability of calcium salts found that there was no difference in the ability of young rats to utilize calcium lactate, carbonate, phosphate, silicate or sulphate, when these were fed in liberal amounts.

Zucker and Barnett (1923) have criticized our interpretation of our experimental data obtained with coconut oil. We stated

that the latter exerted a beneficial effect in rickets produced by our 3143 diet which was excessively rich in calcium, due to the presence in the oil of a small amount of a calcium-depositing vitamin. They base their criticism on the following evidence: When rats were kept for 4 weeks on a diet of flour and a salt mixture containing 2.9 per cent of calcium lactate, rickets was regularly produced. With the addition of 20 per cent of cotton-seed oil or crisco to this diet rickets was still produced. If they lowered the percentage of calcium lactate to 1.5 per cent, the diet without the fat produced rickets, whereas with the addition of 20 per cent of crisco, rickets was prevented, and the same amount of cotton-seed oil did not prevent it but produced a milder degree of rickets. They state that "In this case vitamin action is excluded, but we find the hydrogenated product prevents rickets." The hydrogenation increased the melting point of the oil; and the soaps from crisco are more insoluble than those from cottonseed oil. Their interpretation was "that in the case of crisco more insoluble calcium soaps are formed in the intestine and by precipitating calcium soaps, phosphate in soluble form which would otherwise be precipitated as calcium phosphate is made available for absorption. With a great excess of calcium in the diet containing 2.9 per cent of calcium lactate, the precipitating action of crisco is, however, sufficient to prevent rickets. Now if we turn to coconut oil we can easily see how this fat whose soaps are notoriously insoluble will show a marked rickets-preventing action." The views of Zucker and Barnett appear logical in connection with the kind of experimental data which they discuss, viz., a possible lowering of the effect of an excessive calcium content of the food in relation to phosphorus, the calcium being rendered insoluble in the form of soaps. They did not take into account, however, the statement in the paper of McCollum, Simmonds, Becker and Shipley (1922) that "coconut oil is the only one of the vegetable oils examined which increased the efficiency of the animals in utilizing their *very low* calcium supply." Obviously the formation of insoluble calcium soaps by the fatty acids of coconut oil could not benefit an animal whose diet contained so small an amount of calcium as to be a factor in the production of rickets, as was the case in our experiments.

Zucker, Johnson and Barnett (1922) report the production of rickets in rats on a diet of flour, casein, calcium lactate and sodium chloride, with a trace of ferric citrate. This diet con-

tained an excess of base over acid. When they substituted calcium chlorid for calcium lactate in equivalent amounts they increased the acidity of the diet. When this diet was fed to rats they found the bones to be nearly normal or to show mild rickets. In another experiment they made the diet more acid by adding 2 per cent of ammonium chlorid. These rats did not develop rickets. They also tried the reverse of this experiment, using a diet of flour and egg albumin with a suitable salt mixture. The control rats were given an egg albumin-flour diet to which was added basic potassium phosphate to prevent the development of rickets. To this diet they added 2 per cent of sodium bicarbonate to make it distinctly alkaline. These rats developed rickets. They conclude from these experiments that a diet which from the point of view of balance between calcium and phosphorus should not lead to rickets may do so when there is brought about a situation in which the acidity of the intestinal tract is lessened.

Pappenheimer and co-workers (1922-23) discussed the effects of varying the inorganic constituents of a rickets-producing diet. They found that inorganic salts other than calcium or phosphate seemed to be without influence on the development or prevention of rachitic lesions. They found the results with basic potassium phosphate to be attributable to the phosphate ion and not to the potassium. They found that a diet containing an abundance of phosphate but deficient in calcium leads to changes in the bones which resemble those of rickets but differ from them in the fact that the endochrondal changes are relatively slight or absent. They fed rats a diet deficient in both calcium and phosphate and found that the lesions produced were purely osteoporotic. The swellings of the costochondral junctions were slight or absent and there was no angulation or other deformity. The bones were delicate and fragile rather than pliable. They found that adult rats confined to a diet deficient in phosphate produced a calcium-free osteoid in abnormal amounts about the spongiosa and cortex. The epiphyseal cartilage was not altered as is the case with growing animals.

Production of Rickets by Purified Food Substances.—McCollum and co-workers (1922) produced rickets in rats by diets consisting essentially of purified food substances. Osborne, Mendel and Park (1923) and Goldblatt (1924) also reported the production of rickets in rats on a diet principally composed of purified food-stuffs.

Bethke, Steenbock and Nelson (1923-24) discussed the relations of calcium and phosphorus to growth and composition of blood and bone with varying vitamin D intake (cod liver oil). They presented data on the variation of the composition of these tissues in rats of various ages. They found the blood phosphate and calcium slightly higher in very young than in older animals. On a basal ration containing 105 mg. of calcium and 655 mgm. of phosphorus per 100 gm. of ration, marked increased growth was observed on the one hand with calcium additions, and on the other with the addition of fat-soluble vitamins as found in cod liver oil. This indicates the existence of a quantitative relation between the vitamin and calcium in effecting the assimilation of the latter. They raise the question as to whether or not some of the beneficial results recorded in the literature on the feeding of calcium salts may not have been due to mass action of the calcium counteracting the effects of a vitamin deficiency. They found where no fat-soluble vitamins were added the calcium of the blood was constantly depressed, but apparently the greatest depression took place when the most phosphate was added. They also found that irradiation with ultra-violet light for 10 minutes daily in the absence of fat-soluble vitamins in the diet raised both the calcium and phosphorus in the blood.

Park, Powers and Guy (1924) have extended the studies on the effects of diets on animals in which the calcium was high and the phosphorus low and vice versa. They placed a large series of rats, living in a medium of room light, on a basal diet defective in vitamin D, calcium and phosphorus. In the diet fed two sets of animals the calcium was made high and the phosphorus low. In the diets fed to two other sets of animals the calcium was made low and the phosphorus high. When these diets had been administered long enough for the disproportions between the calcium and phosphorus to become reflected in the blood, cod liver oil was administered. In those animals in which the calcium of the serum was low, cod liver oil caused the calcium to rise to a normal level, restoring a normal equilibrium. In those animals in which the inorganic phosphorus in the serum was low, cod liver oil caused the inorganic phosphorus to rise to a normal level, establishing a normal equilibrium. They interpret their results to mean that cod liver oil acts as a regulator of the mineral metabolism, at least insofar as the calcium and inorganic phosphorus are concerned. Park (1923) stated "one may infer that a function of cod liver oil and also of radiant

energy is to maintain the normal salt equilibrium of the body in the presence of salt combinations of different complexities which are continually being absorbed from the food."

Simonet (1924) confirms the observations of others that rickets may be caused directly by a modification in the composition of the mineral portion of the diet.

Bosányi (1924) has reported remarkable experiments in which he showed that the subcutaneous administration of aqueous extracts of normal bone marrow causes the healing of rachitic bones in the rat. The phosphorus content of the extracts was very small, so the effects could not be attributed to this element. He believes that the failure of the initial deposition of calcium in rickets is due to the stoppage of the biological functions of the bone marrow through which normally a permanent deposition of calcium is insured. Normal functioning bone marrow produces a substance which is transmitted to the tissues to be calcified which renders them capable of calcification. This biological factor in an active state can be extracted with water from the healthy marrow, but this is not identical with the enzyme present in the watery extract. His experiments demonstrate that this factor is not produced in the marrow of rachitic rats.

The fat melted from bone marrow had a slight protection if melted from the red bone marrow, but no effect if from yellow marrow. The marrow wholly defatted protected animals against rickets. When filtered through collodion aqueous extracts of marrow had no anti-rachitic effect. Dialyzed extract had no effect, whereas the gelatinous remainder not filterable had a markedly anti-rachitic effect. Heating to 70° C. did not affect the properties of the extracts, but higher temperatures destroyed it. Extracts were made of liver, spleen, thymus, thyroid and pancreas, and spleen extracts only were found to be anti-rachitic. Extracts of the spleen of a rachitic animal were inactive. Extracts of bone marrow of rachitic rats which had starved four to five days and showed healing were not anti-rachitic. The substance is present only in normal bone marrow. Bosànyi employed the "line test" procedure.

Strontium versus Calcium in Bone Growth.—Stoeltzner (1908) and Lehnerdt (1910) observed that feeding strontium led to the formation of excessive amounts of osteoid tissue in bones. Shipley and co-workers (1922) confirmed this observation and pointed out that although strontium stimulates cartilage cell production it cannot take the place of calcium in making bone.

The administration of cod liver oil to animals whose calcium had been mostly replaced by strontium does not cause any change in the pathological picture produced. This is the only instance which has come under our observation in which cod liver oil has not benefited an animal when confined to a faulty diet affecting the bones. Kinney and McCollum (1923) found that the entrance of strontium into the bones was not inhibited by the presence of calcium in the diet. Lesne, Vaglianos and Christou (1924) confirm these observations on the effect of strontium.

Exercise and Rickets.—Wauschkuhn (1920) reported that dogs and rats exposed to dust-laden and infected air gave no evidence that such conditions are conducive to the onset of rickets. He concluded that domestication or confinement was in no way related to the etiology of rickets. Paton and Watson (1920) reported an experimental investigation with dogs on the etiology of rickets. They emphasized the importance of cleanliness, exercise and outdoor life as important factors in the prevention of rickets. They stated that their results gave no support to the conclusion that rickets is a deficiency disease due to lack of an anti-rachitic factor in the diet. They found (1923) in later work with pups that the limitation of the calcium in the diet proved to be a more important limitation than did lack of vitamin A in the production of rickets. They found cod liver oil to exercise a beneficial effect. Findlay (1922) stated that the most important conclusion to be derived from the experimental and clinical observations is that in some way confinement and defective hygiene are the most potent factors in the production of rickets and that the disease is intimately associated with housing conditions. Diet, he believed, played a subsidiary part, if any, in the etiology of rickets.

Korenchevsky (1921) reported that confinement in small cages does not produce rickets in rats. He found that live cultures of B. perfringens, B. sporogenes and B. bifermentans introduced with the food, or the latter two subcutaneously, produced no visible effects on the development of rickets. He (1922) has prepared a valuable digest of the principal literature on rickets. He described many studies planned to show the calcium and phosphorus requirements of the rat at various ages. His experimental data are too extensive to be digested here. He states, however, that he has produced rickets on a diet deficient only in vitamin A. This no one else has been able to do. He (1923)

found no essential influence of castration on the skeleton of growing rats on a normal or rickets-producing diet.

Composition of Normal and Rachitic Rat Bones.—Buckner and Peter (1921) collected data on the mineral content of white rats. The diet used after weaning consisted of cracked yellow corn which was kept before them at all times. Three times a week stale light bread soaked in fresh whole milk was fed as the morning meal. Twice a week shredded cabbage was fed. Laboratory tap water was used. The following data is taken from their paper.

Average Percentage of Mineral Substances in the Carcasses

Age	P_2O_5		CaO		MgO		K_2O	
Weeks	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
2	0.66	0.87	0.72	0.99	0.04	0.06	lost	lost
8	1.40	1.23	1.12	1.00	0.08	0.07	0.04	0.03
12	1.18	1.12	1.02	0.98	0.04	0.06	0.03	0.04
18	1.22	1.28	0.99	1.09	0.06	0.07	0.03	0.03
40	1.79	1.54	1.47	1.41	0.09	0.08	0.04	0.03

McCann and Barnett (1922) found rachitic rats to contain less phosphorus and calcium per 100 gm. of body weight than normal rats. The reduction was the same whether rickets was produced by a diet high in calcium and low in phosphorus or vice versa. They state that the bones of rachitic rats contain a smaller percentage of total phosphorus than is found in the bones of normal rats. Jackson and Carleton (1923) have compared the weights of various organs of rats suffering from rickets with those of normal rats.

Studies Bearing on Existence of Vitamin D (Calcium-depositing Substance).—It has been pointed out that McCollum, Simmonds, Becker and Shipley (1922) reported an experimental demonstration of the existence of a vitamin which promotes calcium deposition. They found this to be stable in cod liver oil after the oil had been oxidized 12 and 20 hours. This cod liver oil would not cure ophthalmia caused by lack of vitamin A. Dubin and Funk (1923) reported the vitamin potency of cod liver oil not to be appreciably destroyed by a suitable hydro-

genization process. Steenbock and Nelson (1923) confirmed the observation of McCollum and co-workers that aerated cod liver oil would not cure or prevent ophthalmia. They interpret this as lending support to the hypothesis that cod liver oil contains a calcium-depositing factor as well as vitamin A.

Zilva (1922) expressed his belief that it was unnecessary to assume the existence of an additional factor in cod liver oil to explain the deposition of calcium. Goldblatt and Zilva (1923) repeated some of the experiments of McCollum and co-workers and confirmed their observation that the growth-promoting and the anti-rachitic properties were inactivated by heat at different rates in the presence of air. They found a great disparity between the growth-promoting and anti-rachitic potency of spinach. Spinach, when given equivalent to 30 per cent of the diet, did not have the slightest effect in promoting calcium deposition. They confirmed the observation of McClendon that dried spinach cures ophthalmia but does not protect against rickets. They concluded that these observations support the hypothesis of McCollum and co-workers that vitamin A and the anti-rachitic factors are different.

Steenbock, Jones and Hart (1923) found that saponified cod liver oil was equal to untreated oil for maintaining normal values of calcium and phosphorus in blood. They report severe tetanic convulsions in dogs afflicted with rickets which had a normal calcium content of the blood. Apparently, they say, a decreased calcium content cannot be the cause of these convulsions in the animals. It must be remembered that the diagnosis of tetany can only be made as the result of electrical reactions or by a study of the mineral content of the serum. Howland and Marriott (1918) reported that in tetany during the active spasms the calcium content of the serum was invariably reduced and may fall as low as 3.5 mgm. per 100 c.c. of serum. In convulsive disorders other than tetany they found no reduction of the calcium of the serum. The results of Steenbock, Jones and Hart show, from a standpoint of growth, calcification of the bones and maintenance of normal calcium and phosphorus content of the blood, that their extract of saponified cod liver oil is as efficacious as untreated cod liver oil.

In discussing the findings of Howland and Kramer (1920) on the phosphorus and calcium content of the blood in rickets, viz., that in rickets the inorganic phosphorus of the blood serum is much reduced in amount, while the calcium in the same may or

may not be affected, Steenbock and co-workers say: "We have discussed these relations in another publication pointing out the fact that rickets may be prevalent without impairment of the normal calcium and phosphorus content, yet it appears that, with increase in severity of the disease, sooner or later the amounts in the blood stream, especially with relation to the phosphorus, are decreased." Hess and Lundagen (1921-22), Hess and Unger (1922) and Anderson (1924) have also reported that in some cases of rickets no blood changes were demonstrable. Shipley (personal communication) interprets these results as having been made on children or rats in which rickets was in the process of healing. It has never been the experience of the Children's Clinic at the Johns Hopkins Hospital to find active rickets in children or in rats with a normal amount of phosphorus in the circulating blood, unless the rickets was complicated with tetany.

Steenbock and co-workers (1923) confirmed the observation that administration of cod liver oil freed from vitamin A by aeration improved the conditions necessary for normal calcium and phosphorus metabolism.

Lesné and Vagliano (1923) assert that whereas cod liver oil given by mouth even in very small doses exercises curative action upon rickets in rats, when given subcutaneously it was without effect. Kramer and Wilkins (personal communication) state that they have cured rickets in children by subcutaneous as well as by intramuscular injections of an active preparation made from cod liver oil.

Van Leersum (1923) found that injection of hematoporphyrin causes the deposition of calcium salts in rats with rickets produced by diet 3143. The animals were very sensitive to this material and although kept in the dark several died during the course of treatment. This substance renders the animals very susceptible to the visible rays of light.

Shipley, Kinney and McCollum (1924) reported the healing of rickets in rats by a substance extracted from alfalfa leaves by ether, alcohol and acetone. The ether extracts of alfalfa were free from calcium and contained only insignificant traces of phosphorus. Ether also extracted the anti-rachitic vitamin from clover blossoms. Extracts of dry spinach, brussels sprouts, cabbage, celery, tomato and sweet potato were without effect on the rachitic process in the bones. They (1924) found the following oils to have no anti-rachitic value: oils of sandalwood, lemon, orange peel, palm kernel, as well as the oil of spike, of fennel

seed, sperm oil and also the fraction of butter fat insoluble in alcohol, and Japan wax and spermaceti. Animals receiving oil of cloves showed an intensive degree of healing. Huston and Lightbody (1924) reported the cure of rickets by hydroquinol. They also reported that hydroquinol cures the ophthalmia due to deprivation of vitamin A in rats.

Freise and Rupprecht (1921) reported changes in the calcium and phosphorus balances in a well-marked case of rickets following the administration of an extract of carrots. Zucker (1923) showed that an alcoholic and an ether extract of spinach, while it possessed the power to promote growth, did not protect against rickets. McClendon and Schuck (1923) also found spinach to contain no anti-rachitic properties. They attributed the slight protection in rickets by spinach to its content of phosphorus. Kozitschek (1923) found an increase in the calcium retention in rickets on the addition of vegetable juices to the diet. Howland (1923) in his Harvey lecture pointed out that egg yolk possessed anti-rachitic properties. This had been confirmed by Hess (1923), by Casparis, Shipley and Kramer (1923) and by Tso (1924). Eddy, Muller and Heft (1922) report that when the inorganic phosphorus of the diet of rats was replaced by phytin the rats were not protected against rickets although the food consumption remained the same.

Cavins (1924) studied the effect of fasting and refeeding on the calcium and the inorganic phosphorus in blood serums of normal and rachitic rats. After the animals had developed rickets on McCollum diet 3143 they were fasted for short periods and then returned to the rachitic diet. He confirmed the observation of Howland and Kramer that during fasting the inorganic phosphorus of the serum increased markedly. He found that the calcium decreased. He noted that animals returned to the rickets-producing diet after a short fast reverted rapidly to the low blood phosphorus of the rachitic condition. The blood calcium underwent a rapid augmentation, in some cases to above the normal level. He found that there was not such a wide variation of calcium and phosphorus in the serum of normal animals during fasting.

Ederer (1924) found a distinct decrease in the albumin content of rachitic rats which were undergoing treatment with cod liver oil. In the serum of rats showing advanced healing the albumin percentage is below 75 per cent. The individual values ranged mostly between 64 and 75 per cent. He found rachitic rats

to have a somewhat increased albumin content of 84.6 per cent.

Kummer (1920) believes the appearance of tetany following the hypofunctioning of the parathyroids which regulate either the calcium equilibrium of the metabolism of guanidin or more probably both to be due to the disturbance between the calcium and the products of metabolism. The deficiency of calcium deprives the cells of their defense, he believes, and facilitates the entrance into the system of the toxic products of metabolism which give rise to tetany. Pedotti (1921) kept rats on a calcium-free diet and attempted to deplete the body of calcium by potassium feeding. The determinations of the respiratory exchange showed that a lowered basal metabolism resulted from a lack of calcium. He found no histological changes in the thyroid, parathyroid or bone.

Sharpe (1922) reported experiments which showed that the percentage of phosphorus in the blood and in the liver bears no relation to the gain in weight, to the amount of fat in the diet, to the total fat in the blood and liver, or to the age of the animal. In rachitic pups the phospholipins of the blood and the liver were lower than in the non-rachitic pups. He found the amount of phospholipins in the liver to be smaller in proportion to those of the blood in the rachitic than in the non-rachitic animal. Paton (1922) stated that he believed an error in the metabolism of lecithin, possibly in the liver, may be a causal factor in the failure of the bone formation in rickets. He believes that this may in some cases be accompanied by an increased conversion of cholin into guanidin compounds. He explains in this way the association of tetany with rickets. He also does not believe that defective diet and unhygienic surroundings are direct causes of rickets, but believes that an infective agent is still to be considered.

Dodds (1922) reported that the diastatic power of the urine is greatly increased in rickets but it falls to normal during convalescence. He found the fat in the feces to be increased (75 as compared with 20 per cent) in a series of children. He suggests that there may be a pancreatic lesion in rickets leading to a poor production and absorption of fatty acids, and therefore a poor absorption of calcium. He found in most cases of rickets a distinct acidosis with the presence of acetone bodies in the urine.

Luce (1923) found that rats on a calcium-deficient diet showed

a consistent enlargement of the parathyroid glands which was due to hyperplasia and cell hypertrophy. This increased progressively with the length of time the animals were confined to the calcium-deficient diet. Murray (1923) reported that with the occurrence of rickets in pups there are changes in the thyroid which are not associated with the rickets-like changes of calcium deficiency and also that true rickets may attack pups on a diet rich in calcium. Cod liver oil, he found, acted on the thyroid in some way which either prevented destruction or increased the production of the colloid.

Kaufmann, Creekmur and Schultz (1923) assert that in rickets in rats there occur abnormalities of the osseous capsule of the internal ear which are identical with those changes in the long bones which are characteristic of rickets. They believe that these alterations, since they occur in the structures concerned with hearing, may interfere with hearing.

Szily and Edestein (1923) reported the production of lamellar cataracts in young rats nursed by a mother which was fed on McCollum and Simmonds diet 3143. When the litter was large they found the rats to develop cataracts, whereas when the litter was small no cataracts developed. They state that they did not produce rickets on this diet. They found the cataracts to develop usually in the fourth week. Stepp and Friedenwald (1924), attempted to repeat these experiments. They examined the eyes of rats on diet 3143 with the aid of the Gullstrand slit lamp and Zeiss binocular loupe after dilation of the pupils with atropin. These animals uniformly developed rickets but not cataracts. Szily's view was that the cataracts which he produced were due to a deficiency of vitamin A, an excess of calcium and a deficiency of phosphorus in the diet. Stepp and Friedenwald confined animals to a diet very low in vitamin A and phosphorus but relatively rich in calcium. These animals developed both rickets and xerophthalmia but no cataracts. They conclude that it is not possible to demonstrate that diets deficient in either vitamin A or vitamin D with an excess of calcium and low phosphorus could lead to the development of cataracts in young rats.

Stoeltzner (1920) believed the favorable action of cod liver oil to be due to its content of oxy-acids. Sjollema (1922) found that in rabbits cod liver oil caused a diminishing of the secretion of calcium by the kidney as well as by the intestinal tract. When a diet rich in calcium was given the quantity of the feces as well as their content of calcium decreased if cod liver oil was given.

He concluded from his experiments that the organism can give off larger quantities of calcium than are taken up, and that the secretion of phosphoric acid runs nearly parallel with the calcium. Allison and Brooks (1921) observed that non-use has no effect on the chemical composition of the matrix in dogs. An entire bone may lose more than 25 per cent of its mineral constituents in 24 days of non-use. This they attribute to loss of bone matrix and not to change in composition.

Diet of Mother and Susceptibility of Young to Rickets.—Hess and co-workers (1923) pointed out that the term stock diet should not be used indiscriminately and when work is reported this diet should be described. They believe that the character of the food of young rats during the first four weeks of life is of great importance regarding the later susceptibility to rickets. They found that cod liver oil or the vitamin contained in it was not transmitted through the mother's milk in sufficient amounts to afford protection to the young, but cod liver oil afforded great protection when fed during the pre-experimental period.* They believe that the young animal stores the protective substance in its body which it derives from the food after it begins to depend upon the stock diet, but is not protected through the mother. Hess (1923) reports that a few tests on human beings indicated that an infant cannot be regularly protected against rickets by giving cod liver oil to the mother during pregnancy. Hess and Weinstock (1924) again assert that cod liver oil, when given to the mother during pregnancy, cannot be relied upon to protect the offspring against rickets. They reiterate their belief that the active principle of the oil is not excreted into the milk in adequate amounts to protect the young, but if the young are fed cod liver oil during the last half of the nursing period when they are supplementing the milk with the stock diet, protection is afforded. Korenchevsky and Carr's (1923) experience demonstrated that when mother rats were fed during pregnancy or lactation on a diet deficient in vitamin A or calcium there was a marked increase in the disorders of general nutrition with rachitic changes in the skeleton of the offspring kept on a minus A diet. The same was true when the mother was also kept on the same insufficient diet during lactation. They state that feeding the mother during lactation on a diet rich in vitamin A and calcium hinders for a considerable time the disorganization of general

*Unpublished experiments in our laboratory have convinced the authors that vitamin D is transmitted through the milk of the rat.

nutrition and the development of obvious rickets in the offspring kept on a diet deficient in fat-soluble vitamin. These experimental data point to the possibility of influencing the occurrence of rickets and general disorders of nutrition in the offspring through the diet of the mother.

Chemical Studies on Active Principle in Cod Liver Oil.—Zucker, Pappenheimer and Barnett (1921) stated that the anti-rachitic principle was found in the ether-soluble, unsaponifiable fraction of cod liver oil after alkali hydrolysis. They state that it is not an organic base of the type described as occurring in cod liver oil nor is it a cholesterol. They believe it may be a sterol related to cholesterol. They found that the fatty acids of cod liver oil are entirely inactive in curing rickets. Zucker (1922) reported that a good yield of crude product of the anti-rachitic vitamin could be obtained by extracting cod liver oil with 95 per cent alcohol. The mixture of fatty acids in a small amount of oil and other substances was saponified with NaOH. When the calcium soaps were precipitated from aqueous solution the unsaponifiable matter including the active substance was precipitated with the soap. He stated that from the calcium soap acetone would extract the active material. The preparation which Zucker made was active in dilutions of 1:1000. He reported that a single dose brought about the healing of rickets in about the same manner as a succession of small doses. He has not described the method of making these tests. The preparation was found to be free from vitamin A. Zucker and Barnett (1923) subjected the following substances to processes similar to those by which the anti-rachitic substance of 1:1000 activity was obtained from cod liver oil: butter, coconut oil, spinach, carrots, pig's liver and sheep adrenals. They did not obtain as active an extract from any of these as from cod liver oil. They found it would take 4 kilograms of spinach or carrots to furnish as much vitamin D as is furnished by one teaspoonful of cod liver oil. Zucker and Matzer (1924) found that when a solution of the active principle of cod liver oil is put into olive oil or glycerol, and injected subcutaneously into rats, it does not produce healing or change the pH value of the feces provided measures are taken to prevent the licking of the site of injection. Kramer and Wilkins (personal communication) have cured rickets in children by the intramuscular injection of an extract of cod liver oil prepared by Dr. Wilkins. The chemical changes in the blood before treatment were such as to indicate that the process was

active and this was confirmed by radiograph of the extremities. The increase in the calcium and inorganic phosphorus concentrations of the serum was similar to that following the oral administration of cod liver oil. The experimental rickets of rats responded in a similar manner to the subcutaneous injection of the extract.

Changes in the Composition of the Blood in Rickets and Tetany.—Howland and Marriott (1918) discussed the theories as to the causation of infantile tetany. Tetany has been referred to: (a) to diseased condition of the parathyroid glands; (b) to the character of the food; (c) to intoxication by calcium; (d) to intoxication by guanidin, or methylguanidin; and (e) to lack of calcium. They stated that the evidence in 1918 failed to support most of these theories, although there had been in the literature for a hundred years satisfactory descriptions of infantile tetany. They pointed out that normal calcium of the blood is 10 to 11 mgm. per 100 c.c. of serum. In some cases of rickets they found a lowering of the calcium to about 8 mgm., although in some cases of rickets the calcium remained normal. In tetany during the active symptoms the calcium content of the serum was invariably reduced and might fall as low as 3.5 mgm. In convulsive disorders other than tetany they found no reduction of the calcium of the serum. They found calcium chlorid given by mouth caused a prompt relief of the spasmodic symptoms.

Howland and Kramer (1921) reported studies on the determination of the inorganic phosphorus of the serum. Their results represent the orthophosphoric acid content of the serum, the only form of phosphorus which can react with calcium to form tertiary calcium phosphate. They believe there can be no doubt that the inorganic phosphorus represents a definite chemical entity since it is present in nearly constant amounts in the same individual as well as in normal individuals of the same age. Their studies show that in rickets there is a marked decrease in inorganic phosphorus in the serum. To this deficiency they ascribed the failure of calcium deposition. Non-rachitic infants and young children have between 10 and 11 mgm. of calcium and about 5 mgm. of inorganic phosphorus per 100 gm. of serum. All of the children under two and a half years with 3 or less than 3 mgm. of phosphorus had active rickets. When cod liver oil was given to these children the phosphorus content returned to normal. This rise in the phosphate content of the serum was accompanied by healing of the lesion of rickets.

Kramer, Tisdall and Howland (1921) found the calcium of the serum in a large series of cases of rickets associated with tetany to be about 5.4 mgm., or roughly half the normal. In 10 cases of active tetany the average sodium content was 325 mgm., which is about normal. The highest concentration was 337 mgm., and the lowest 320 mgm. In a similar number of cases of active tetany the average potassium concentration of the serum was 24.3 mgm., which is about 25 per cent above the average of 19.3 mgm. The magnesium concentration was 1.9 mgm., the average for normal infants being about 2.5 mgm. It is obvious from these figures that the essential change is in the calcium and that this change is constant. The sodium is normal in amount; there may be a slight increase in the potassium; the concentration of magnesium is essentially unaltered. The phosphorus content is usually within the limits of normal or slightly above normal. An increase in the inorganic phosphorus does not seem to be responsible for infantile tetany. The ratio Na: K::Ca:Mg is increased. This is almost wholly due to a decrease in the concentration of calcium. The increased irritability of the neuromuscular mechanism which is the essential phenomenon in infantile tetany they believe to be due to the diminution in the concentration of calcium in the serum.

Von Meysenbug (1921) concluded from his studies that there appears to be no change in the proportion of diffusible calcium in the serum in human rickets or in experimental dog tetany. These results are out of harmony with those of Kramer and Howland. Kramer and Howland (1922) report that the normal concentration of calcium in the serum of rats is from 9.5 to 10.5 mgm., and of phosphorus from 7.0 to 8.5 per 100 c.c. of serum. These values are not increased by changes in the diet or by ultra violet light. In animals fed diets very low in calcium or phosphorus the concentration of these elements in the serum could be diminished by as much as 50 per cent. Subsequent addition of fish liver oil to the food brought the values back to normal. The phosphorus could also be raised by illumination with light of less than 3000 A°. They also found that a similar effect could be obtained by starvation for a few days.

Kramer, Casparis and Howland (1922) treated 5 children showing clinical evidences of rickets, confirmed by x-ray examination of the bones, by systematic exposure to the rays of a mercury quartz lamp. This was followed in every case by a healing of the rachitic process in the bones. Before treatment

was begun the inorganic phosphorus of the serum was low, ranging from 2.7 to 3.2 mgm. The changes in the phosphorus of the serum following the light treatment were the same as when the children were given cod liver oil. The pigmented skin of Negroes did not interfere with the action of the light rays. They did not require more intensive treatment than did the whites to bring about the effects. This work has been confirmed with sunlight by Kramer and Boone (1922).

Brown, Tisdall and co-workers (1922) reported two cases of rickets accompanied by tetany in breast-fed infants. The diet of the mothers during lactation was poorly constituted in that it lacked sufficient vitamins and certain inorganic elements. The breast milks were abnormal in their ash contents. One showed a low calcium and a high potassium and phosphorus. The other was low in calcium and phosphorus. The blood serum of the babies had an abnormal concentration of calcium and inorganic phosphorus.

Telfer (1922-23) found no negative balances of either calcium or phosphorus in rickets. He believes there is no support for the theory that excessive decalcification is the cause of bone softening in rickets. He suggests that defective absorption of calcium from the intestine, due possibly to the result of some alteration in the gastro-intestinal functions, cannot be excluded as a factor in the causation of bone softening in rickets.

There are several explanations for the diminution in the retention of calcium and phosphorus in rickets. One is that calcium and phosphorus are not absorbed from the intestinal tract. On this supposition it is likely that either the digestive functions are disturbed in some peculiar way or else the combination of food substances is such that insoluble products incapable of absorption are formed. Another possibility is that the calcium and phosphorus are absorbed but on account of abnormal conditions in metabolism they cannot circulate in the blood; they are either driven out of the body or are used to carry some other substances. Under this latter supposition it is necessary to suppose the primary disturbance to be in the metabolism. In either case it seems altogether probable that the deposition of lime salts in the bones is determined chiefly by the products of the concentration of calcium and phosphate ions in the circulating fluid, as Howland and Kramer have indicated. Their work shows that when the concentration of calcium and phosphorus increases sufficiently the deposition of lime salts occurs.

Howland and Kramer (1922-23) found that in uncomplicated cases of rickets the concentration of calcium in the serum is normal or nearly so, whereas the phosphorus concentration is regularly low so that the product of the two concentrations is at or below 30. In tetany the product is also low on account of the striking reduction in the calcium. In all of these cases rickets was present. After cod liver oil or ultra violet ray therapy the phosphorus rises so greatly as to make the product two or three times what it was before. The graph (Fig. 23) taken

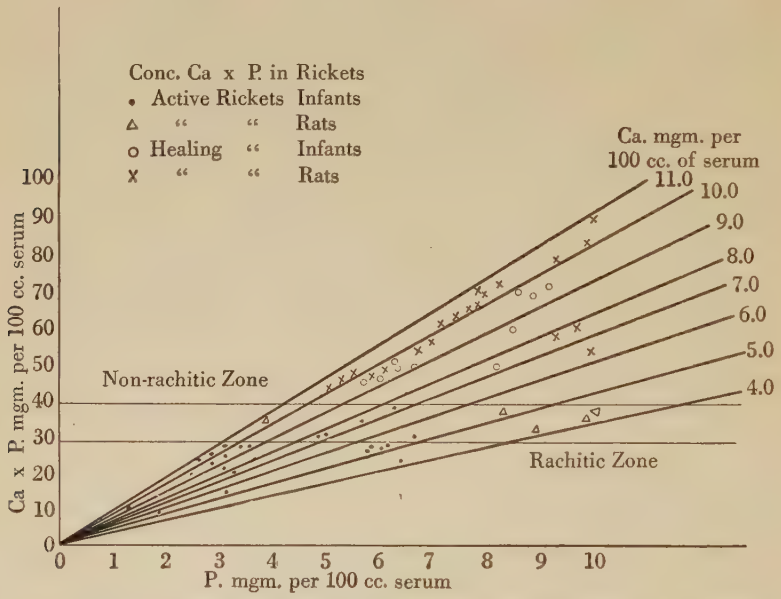


FIG. 23.—Graph taken from paper by Howland and Kramer, Monatsschr. f. Kinderheilkunde, 1923, xxv, 279.

from their paper was constructed from figures for the concentration of calcium and inorganic phosphorus both of children and of rats. This illustrates clearly the significance of the products of these concentrations. On the abscissa are marked the concentrations of phosphorus; the oblique lines represent the concentrations of calcium and the products may be read off from the ordinates.

The cases of active rickets occurring in children are represented by a circle, and those in rats by a triangle. Healing is indicated by a dark circle or by a dark triangle. It will be noted that

below the horizontal line corresponding to the figure 30 on the ordinate all the triangles and circles are light. Above 40 they are all dark. Between 30 and 40 only one is dark. This graph is to be interpreted that when the product of calcium times phosphorus is below thirty, rickets is to be expected, between 30 and 40 it is probable. When the product is above 40 either healing is taking place or rickets is entirely absent.

Orr, Holt, Wilkins and Boone (1923) report metabolism studies which show that infants with active rickets fail to retain phosphorus and calcium, although adequate amounts are present in the diet. On irradiation with ultra-violet rays there was a very marked increase in the retention of these elements. They state that it seems justifiable to conclude that the ultra violet rays act by increasing the absorption of calcium and phosphorus from the intestine. The healing effects of both cod liver oil and the ultra-violet ray appear to be due to their ability to promote absorption from the intestine.

Clark (1923) found that the diffusible calcium of the blood serum measured by dialysis in collodian sacks against physiological sodium chlorid was higher after the serum had been exposed to radiations of the mercury lamp. The diffusible calcium in rabbit serum rose from 38 to 58 per cent and in dog serum from 44 to 53 per cent.

Hess and Matzner (1923) observed no relationship between the inorganic phosphate content of the serum and the occurrence of rickets during the first year of life. They found the phosphate content of the serum of the mother to be invariably lower than that of her infant at birth, and the same was true of calcium but to a lesser extent. These results are out of harmony with those of Howland and Kramer.

Hess, Calvin, Wang and Felcher (1923) report in their studies on rickets that the phosphorus or calcium or both may be moderately lowered. From their studies of the blood picture in rickets and in rickets associated with tetany they believe these are two phases of the same disorder. They found in severe rickets the phosphorus to be markedly lowered even in those babies exclusively breast-fed, whereas the calcium was not always lowered. In 40 per cent of the tetany cases severe rickets was present. They confirm the studies of Howland and Kramer that in rickets accompanied by tetany the calcium is reduced, whereas the phosphorus may or may not be reduced.

Kaneko (1924) found that the hemoglobin content of the

blood in rickets was normal. The red corpuscles were slightly decreased in number, as were also the neutrophylic polymorphoneuclear leucocytes.

Blood Studies in Fractures.—Tisdall and Harris (1922) reported studies on the inorganic content of serum of 42 persons with fractures, ranging in age from 4 months to 44 years, and of 11 persons between the ages of 20 and 57 years. They found the inorganic phosphorus content of normal serum from birth to 20 years to be remarkably constant at 5.6 mgm. per 100 c.c. of serum. At 20 years of age the phosphorus content drops sharply to about 3.75 mgm. and remains constant throughout adult life.

During the period of union of fractures in the adult they found the phosphorus content of the serum to be raised to a level of about the same as that present in childhood. They found a close parallelism between the concentration of the inorganic phosphorus in the serum and the deposition of new bone, both during the process of growth and of repair. Eddy and Heft (1923) confirmed the observations of Tisdall and Harris. Moorhead and co-workers (1923) also reported studies on the phosphorus and calcium concentration of the serum of patients during the period of fracture union.

Petersen (1924) reported an experimental study of ununited fractures with especial reference to the inorganic bone-forming elements in the blood serum. He applied the principles elaborated by Howland and Kramer, and by McCollum, Simmonds, Shipley and Park (see p. 396) in their studies of experimental rickets in the rat. He found it possible by dietetic management to lower the phosphorus content of the blood of dogs to a point where the product of calcium times phosphorus was less than thirty. He concluded from his study that in the healing of fractures a definite relationship exists between the concentration of the inorganic bone-forming elements in the serum and the rate of repair. If the phosphorus and the calcium times phosphorus product are again raised to their normal level the fractured bones will unite. This condition he was able to establish by dietetic management. He (1924) applied these principles to a clinical study of ununited fractures. He gives the following case as illustrative of what sometimes occurs in human fractures. On July 19, 1921, a man, aged 47, was struck a glancing blow on the wrist, fracturing both radius and ulna. The arm was put into a splint. On August 22, 1921, the splint was removed, but no union

had taken place. On January 16, 1922, an open reduction was performed and the fragments were wired. This did not result in union. On July 24, 1922, another operation was performed. This time a Lane plate was used. Infection followed and the plate had to be removed. On December 11, 1922, the patient had a third operation, and on March 23, 1923, another unsuccessful attempt was made for union. The patient came to the Johns Hopkins Hospital in November, 1923, for further study. The patient was operated upon on November 7. The ends of the fragments were freshened and wired into position. The calcium and phosphorus of his serum were found to be 10.8 and 2.225 mgm. per 100 c.c. of serum respectively.

Sample menus which the patient received are as follows: Dinner: soup, creamed salmon, baked potato, stewed prunes; Breakfast: cereal and bacon; Dinner: soup, meat loaf, mashed potatoes, string beans; Supper: soup, creamed potatoes and stewed apricots; Breakfast: cereal and bacon; Dinner: soup, roast veal with dressing, mashed potatoes, buttered peas and ice cream; Supper: creamed potato, beet salad, bananas; Breakfast: cereal and bacon; Dinner: soup, frankfurters, potatoes, buttered beets; Supper: soup, creamed chipped beef, baked potato, stewed apricots; Breakfast: cereal and eggs; Dinner: soup, stew with crust, potatoes, buttered string beans; Supper: soup, hashed brown potatoes, stewed pears; Breakfast: cereal and bacon; Dinner: soup, corned beef and cabbage, potato, chocolate pudding; Supper: soup, potatoes, apple sauce; Breakfast: cereal and hash; Dinner: soup, Hamburg loaf, creamed potatoes, corn. Bread and butter was included with all menus and milk was provided for the breakfast cereal. In addition this patient received daily 2.0 gm. of calcium carbonate, 1.0 oz. of cod liver oil, 500 c.c. of buttermilk, orange juice or raw vegetables as cole slaw. In addition he was also given calves' liver three times a week.

No union took place during the first 6 weeks following the operation. During this period the product of the inorganic calcium and phosphorus remained below thirty. With this diet the phosphorus gradually increased. On January 2 the calcium was 10.3, and the phosphorus 3.75, and the product 38.1. The mercury vapor quartz lamp was substituted for the cod liver oil, since it was thought that this would raise the phosphorus still further. On February 18 the calcium was 10.4 and the phosphorus 4.0, and the product 41.6. On May 1 the radius had united and the ulna was uniting. He cites other instances where

cod liver oil was used without light treatment and where union took place. Dr. Petersen suggests that cases of non-union may be essentially a dietary problem.

Effect of Light in Prevention and Cure of Rickets.—There has long been a belief among clinicians that sunlight, calcium-rich foods and cod liver oil are of value in the treatment of diseases, especially rickets and tuberculosis.

Palm (1890) as a result of a topographic study of the incidence of rickets believed that sunlight should be looked upon as a therapeutic agent. Buchholz (1904) published a paper on the treatment of rickets by means of light and certain other diseases occurring in children. He used the "Glühlicht" (Kellogg) which, he stated, was poor in chemically active rays. The heat rays were in part absorbed by a glass filter. Buchholz believed that the action of the lamp depended in part upon the heat rays and in part on the light rays. He treated 16 rachitic children with light and all showed general improvement. His work, however, did not attract the attention which it deserved.

Neumann (1909) observed that rickets and tetany did not occur in Arosa (1740 meters) but occurred frequently at Davos at an altitude of 1560 meters. At Arosa the children lived most of the time out-of-doors, whereas at Davos most of them lived under crowded conditions found in the cities.

Raczynski (1912) wrote: "It is the sun which plays the principal rôle in the etiology of rickets." He gave the first proof of the favorable influence of light on mineral metabolism by an experiment on puppies. One puppy was reared in the dark, the other in the sunlight, and both were suckled by the mother. At the end of 6 weeks Raczynski found that the calcium oxid and the phosphorus pentoxid in the body of the puppy reared in darkness was very much less than that of the one reared in sunlight. In commenting on his studies he stated: "It is possible to assume that the lack of action of sunlight by influencing in so unfavorable a manner the assimilation of calcium oxid in the young organism is one of the causes of rickets." Huldschinsky (1919) reported that the ultra-violet ray exerted a curative action on rickets. Winkler (1918) reported the favorable effects on the treatment of rickets with x-rays. Putzig (1920), Huldschinsky (1920), Riegel (1920), Erlacher (1921), Mengert (1921), and Hess and Unger (1921) extended these observations and established the therapeutic value of light. Sachs (1920-21) and Huldschinsky (1920) reported the cure of tetany with light treatment.

Hess and Unger (1920) reported the results of treatment of 5 rachitic children with the rays of the mercury vapor lamp over a period of 3 months. The entire body was exposed for 20 minutes daily so that it became brown as if tanned by the sun. This treatment, they asserted, did not lead to a definite improvement in the rickets nor did it benefit their general condition. Violet rays, they stated, cannot be considered the equivalent of heliotherapy. They (1921) further studied the effects of ultra-violet and sun's rays and observed definite improvement and cure of infantile rickets.

Hess and Unger (1921) were the first to demonstrate by means of the x-ray that sunlight alone possesses the same curative action as the light of the mercury vapor quartz lamp in human rickets. They exposed 5 infants with rickets to direct sunlight for periods of one-half to several hours daily when sunlight was available. The general condition of the infants as well as the condition of the bones was benefited. Calcification at the cartilage-shaft junctions of the bones of the extremities occurring in the course of treatment was determined by the X-ray. Hess and co-workers (1921) reported the protection of rats against rickets by sunlight.

Shipley and co-workers (1921) reported the following experiment on the effect of sunlight on rickets in rats. Two groups of rats were fed diet 3143. One group was kept under laboratory conditions. A second group was exposed to sunlight an average of 4 hours daily for 62 to 67 days. They were then killed. Those kept in the laboratory all showed gross and microscopic appearances of rickets. The rats exposed to sunlight showed no evidence of rickets. Although sunlight prevented rickets the bones of these animals were not of excellent quality. The exposure to the sun's rays did not entirely compensate for the defects in the diet. The animals remained undersized. The bones, though completely calcified, remained thin. In commenting on these studies the authors stated: "As the result of the gross and histological examinations made on the rats fed the rickets-producing diet 3143, but exposed to sunlight, it is possible to say that the changes produced by sunlight in the skeleton do not differ in any important respect from the changes produced when the animals are kept in room light but on a diet supplemented by cod liver oil. Cod liver oil contains something which is essential for optimal cellular function. Light also contains something which is essential for optimal function. Cod liver

oil or light, when made available to an organism previously deprived of either, permits the organism to put into successful operation adaptations or defense mechanisms which otherwise would have been ineffectual. Neither cod liver oil nor light meets the defects in the composition of the diet directly by supplying to the body either calcium or phosphorus, but meets them indirectly by so raising the potential of cellular activity as to secure the most efficient utilization possible of those substances available in the body which are directly or indirectly concerned with ossification and calcification."

Hess, Unger and Pappenheimer (1922) reported that the same protection can be afforded rats by exposure to the sun's rays as is afforded by adding 0.075 gm. of phosphorus to the diet in the form of dipotassium phosphate. Hess and co-workers (1922) concluded that rays as long as 334μ have little or no protection against rickets. They believe that the active rays begin in the neighborhood of 310μ. Powers and co-workers (1922) confirmed the prevention of rickets in rats with the mercury vapor quartz lamp. Goldblatt and Soames (1922) observed that the growth of rats is stimulated by the mercury vapor lamp when a diet deficient in vitamin A is fed. They stated that irradiation is no substitute for the vitamin but in some way economizes its action. If the diet was too low in vitamin A the bones were not normal. Sheets and Funk (1922) fed rats a diet deficient in vitamin A and exposed them daily for 3 to 5 minutes to the ultra-violet rays. They found the rays to prevent rickets but not xerophthalmia.

Shipley (1922) reported that animals exposed to the direct rays of the sun or to iron-chromium, cadmium, or mercury vapor quartz lamp were enabled to compensate for a faulty calcium: phosphorus ratio. He found the shorter ultra-violet rays (about 2100 A°) to have the greatest anti-rachitic effect. The same changes in the blood calcium and phosphorus were induced under the influence of these rays as by giving cod liver oil. Goldblatt and Soames (1923) placed two litters of rats on diets containing optimal amounts of calcium, phosphorus and vitamin A. In the diet of one group vitamin A was supplied by cod liver oil, in the other it was supplied by butter fat. Half of each litter was kept in the dark, the other in daylight, with an addition of daily irradiations for 20 minutes under the mercury vapor lamp. It is interesting to note that since both groups were given an adequate

diet no difference was to be found between the two groups histologically or in the calcium content of their bones.

Hess and Weinstock (1923) state that the spectrum contains not only rays which can prevent or cure rickets but also rays which are able to neutralize or inhibit the effects of those which are beneficial. When the light of a mercury vapor lamp was filtered through glass of known transmission wave lengths they found that above 302-313μ had no curative action. They interpret their evidence to mean that the longer wave lengths (the visible rays of infra-red) inhibit the curative action of the ultra-violet rays. Eckstein (1923) demonstrated that the growth of rats is independent of the quality of natural light (red, blue, violet or gray), and of its intensity. Artificial ultra-violet light he found produced a retardation in the growth of these animals. Animals on a vitamin-free diet died sooner after ultra-violet radiations than those not exposed to the rays.

Steenbock and Black (1924) report that rat rations irradiated by the mercury vapor quartz lamp can be activated so as to make them growth-promoting and bone calcifying to the same degree as when the rats are irradiated directly. The activation takes place when the ration is irradiated in an open dish or in a stoppered pyrex or quartz flask filled with air or carbon dioxid, but not in a brown bottle. The activation is not destroyed by subjecting the ration to a vacuum, heating it for 45 minutes at 96° C, or letting it stand for 24 hours at room temperature. They confirm the observation of Goldblatt and Soames (1923) in finding that liver taken from irradiated rats is growth-promoting, whereas the liver from non-irradiated rats is inactive. The same was found true of the lung and muscle tissue. Inactive muscle, after removal from the body, exposed to the radiations of the lamp was found to have become activated, being both growth promoting and bone calcifying. Liver treated the same way also promoted bone calcification. The activity of the liver taken from irradiated rats was not destroyed by drying at 96° C for 24 hours and then keeping it in a stoppered bottle for 2 months.

Kugelmass and McQuarrie (1924) discuss the photoactivity of substances curative of rickets and the photolysis of the oxy-products by ultra-violet radiation. The data presented show that the substances curative of rickets upon oxidation blacken photographic plates through quartz but not through glass screens. Ultra-violet light passes through quartz but not through glass.

They tested the following substances reported to be curative of rickets, viz.: cod liver oil, non-saponifiable fraction of cod liver oil, oxidized cod liver oil treated so as to destroy vitamin A, egg yolk, bile, hydroquinol, and sperm oil. The following substances not curative of rickets, viz., linseed oil, peanut oil, cottonseed oil, lard, the saponifiable fraction of cod liver oil and also crude oil, albolin, and glycerin, when oxidized did not affect the photographic plate through either quartz or glass. They also state that "The experimental data further show that the oxidized substances curative of rickets when exposed to ultra-violet radiation liberate oxygen. Therefore the photochemical reaction appears to be reversible and may be expressed by the equation, Oxygen + Rickets-curing Substances = Oxy-substances + Ultra-violet Rays.

Hess (1924) states that attempts to prevent rickets in rats by means of fluids which contain radium failed. Water to which radium bromid had been added was fed daily but without effect. This was likewise true with regard to linseed oil to which minimal amounts of radium had been added. Subcutaneous injections in water were also without effect. He found that cottonseed oil when rayed for an hour with the mercury quartz lamp at a distance of one foot acquired anti-rachitic potency. Linseed oil was also made anti-rachitic by ultra-violet rays. Mineral oil irradiated in the same manner gave a negative result.

MacCallum (1924) states that he found that the vapor arising from cod liver oil will produce a picture on a photographic plate exposed to it in the dark if the plate is protected by some pattern. This is definitely due, he believes to direct chemical contact with the vapor and not to radiation because there is no effect if a quartz plate is interposed.

Langworthy (1924) observed that ripe tomatoes possess radioactivity. The values obtained were equivalent to 3 or 4 times that of the water of Washington, D. C. The radioactivity became less apparent as the product aged.

Luce (1924) discussed the relation of the degree of illumination to which a cow is exposed as regards the anti-rachitic and growth-promoting vitamins in her milk. She says that the anti-rachitic value of the milk depends upon whether or not the cow is kept in pasture or whether she is stall-fed in the dark. Milk from stall-fed animals is very inferior to that from animals in sunlight in its vitamin D content.

Boaz and Chick (1924) also discussed the influence of diet and

management of the cow upon the deposition of calcium in rats receiving a daily ration of milk in their diet. They state that the influence of cod liver oil upon calcium deposition is not to be attributed to specific properties peculiar to that substance, but to a high content of the fat-soluble vitamin, since a similar deposition can be demonstrated in the case of cow's milk, the degree depending upon the diet of the cow and the amount of sunlight to which she is exposed.

It will be clear that in the development of rickets several factors operate. Diet is of fundamental importance, but hygienic conditions, especially sunlight, is at times a determining factor. The absence of rickets among the primitive Eskimo demonstrates clearly that the diet of the mother during prenatal life and the nursing period can protect the child against rickets in the absence of sunlight. Many human infants are born predisposed to rickets because of faulty diet of the mother. The long period of slow growth in the human infant puts it in jeopardy in greater degree than is the case with the young of domestic animals since conditions favorable to rickets can act over a longer time. Outdoor life in winter is less effective than in summer because in winter the child in the temperate regions is so heavily clothed that it is but little exposed to the sunlight.

The experiments reported in this chapter demonstrate that rickets can be produced experimentally in rats by certain unfavorable relations between calcium and phosphorus and an inadequacy of the vitamin D. Since rickets is a disturbance of calcium and phosphorus metabolism, disturbances of digestion, which interfere with the assimilation of these elements, undoubtedly contribute to the development of this disease in children.

CHAPTER XXI

CALCIUM ASSIMILATION IN FARM ANIMALS

Green Food versus Dry Food and Calcium Assimilation.—Steenbock and Hart (1913) presented data which showed that there were marked differences in the amount of calcium eliminated in the feces of a milking goat when the animal was changed from old, dried roughage to green pasture. During the earlier part of the experiment when the animal was on dry feed there was progressive increase in the content of calcium in the feces. The goat became greatly debilitated, and was placed on June grass pasture and given oats in addition. It rapidly recovered with increase in weight. After 2 weeks it was returned to the metabolism cage for a new calcium balance record. After the period of green feeding the fecal calcium elimination was so reduced that the animal gave a positive balance as compared with a high fecal calcium output, and negative calcium balance on the dry ration. The interval of green feeding, therefore, enabled the animal to again assimilate the calcium of the dried feeds, which it had become unable to do after confinement to the dry feed for a considerable interval. The reason for this was not at that time apparent, but it was evident that there was something about the green vegetable food which favorably influenced skeletal storage of calcium, and made possible a better assimilation of this element from the intestine.

Forbes and associates (1916-18) have made many important contributions to the subject of calcium metabolism by showing that high milking cows receiving rations that were supposed to provide an ample intake of calcium may, nevertheless, eliminate a larger amount of calcium than was ingested. They found that the addition of calcium salts to a ration of grains, dry alfalfa hay and corn silage, did not lead to the establishment of positive calcium balances.

Meigs, Blatherwick and Cary (1919) extended these studies by showing that a dry but pregnant cow probably does not assimilate sufficient calcium from a calcium-rich ration such as

dry alfalfa hay, corn silage and a grain mixture to keep her in positive balance, but actually transfers calcium salts from her skeleton for fetal skeleton building.

Hart and co-workers (1920) reported experiments with cows and goats which showed that milking animals receiving grains and dry oat straw are brought into a decidedly negative calcium balance. There was a larger fecal calcium indicating a failure of calcium assimilation. With milking goats fed a dry ration there was a negative balance of 1.6 to 2.3 gm. of calcium oxide per day but when the dry straw was replaced by an equivalent amount of fresh green material the negative balance was 0.6 gm. per day. They concluded that there is something in green feeds which affects calcium assimilation and therefore milk production.

Hart, Steenbock and Hoppert (1921) reported experiments demonstrating that there is something in fresh green oats as compared with dry oat straw, which increases the amount of calcium assimilated. They found that oat hay dried out of direct sunlight, but in a fairly well lighted attic, seemed to retain the property of the fresh green oats. They also found that 120-240 c.c. of orange juice per day did not have a consistent effect on calcium assimilation. Raw cabbage was found not to be superior to dried cabbage, so the possibility that vitamin C had something to do with calcium assimilation was eliminated. They found that 5 to 10 c.c. of cod liver oil consistently changed a negative to a positive calcium balance. Their data were not extensive enough, they stated, to warrant final conclusions on the ability of butter fat to favorably influence calcium assimilation.

Hart, Steenbock, Hoppert and Humphrey (1922) found that liberally-milking cows were maintained in positive calcium balance when dry alfalfa which had been cured under caps was used as a roughage and as the main source of calcium, but supplemented with corn silage and a grain mixture. The alfalfa hay consumption was 10 lbs. per day. These investigators had formerly not had a calcium balance with dry alfalfa fed to dairy cows and state their belief that possibly there is a variation in the kinds of alfalfa. Forbes did not secure calcium retention with dry alfalfa as the principal source of this element. Hart and co-workers also found that with fresh green alfalfa there was a more effective storage of calcium than when the animals were on the dry plant. They noted a positive phosphorus balance when the animals were in a positive calcium balance. Hart, Steenbock and Bethke (1922) reported experiments using alfalfa

which was cured in a windrow exposed to the air and light for 4 days. This alfalfa did not bring about calcium and phosphorus equilibrium in milking cows. They were unable to explain why the alfalfa used in the preceding experiment which was cured under caps gave a positive calcium balance, whereas that cured in windrows gave negative balances. They state that in drying a difference in the destruction of the vitamin assisting in calcium assimilation apparently took place. Supplementing timothy hay with bone meal did not result in establishing positive balances or even equilibrium although the losses were reduced as compared with the unsupplemented timothy hay.

Dispersion of Calcium Salts and Calcium Assimilation.—Hunt and Winter (1923) reported experiments with goats which they interpret to mean that although there may be a vitamin in green plants which assists in the assimilation of calcium, a part of the difference between green and dried plants is due to the physical properties (degree of dispersion) of calcium of the cell and cell wall.

Hunt and co-workers (1923) determined balances for sodium, potassium, calcium, magnesium, sulphur, chlorine, phosphorus and nitrogen in dry and lactating goats fed the following series of food-stuffs: (1) dry roughage (millet hay) and grain; (2) green roughage (millet) and grain; (3) dry roughage, grain and bone flour; (4) green roughage, grain and bone flour. In general, they found the mineral metabolism of the goat to be similar to that of the cow. The calcium and chlorin content of goat milk was found to be higher than that of cow milk. Their results showed that as a rule the digestibility of protein, carbohydrate and ether extract is increased in changing from a dry ration to one containing green roughage. They presented evidence in harmony with that of Hart and co-workers that the calcium in a green roughage ration was better assimilated than that of a ration of dry feeds alone. Their interpretation, however, was different from that of Hart. The former believed that the calcium in green food is more highly dispersed and hence better assimilated than that of dry feeds.

Hunt, Winter and Miller (1923) working on the hypothesis that most of the calcium in whatever combination it may be in the cells of green plants is in a highly dispersed form, made a starch paste with a known solution of calcium chlorid (4N). An equal volume of Na_3PO_4 of the same strength as the calcium chlorid was added. The starch acted in a slight degree as a

protective colloid for the calcium ions, and the final product, $Ca_3(PO_4)_2$ was left in a highly dispersed form. This starch paste was added to a ration of grain and dry timothy hay, and was fed to two milking goats. The grain mixture used consisted of 100 parts of ground corn, 100 parts of ground oats, 50 parts of wheat bran and 30 parts of linseed oil meal. The proportion of hay to the grain mixture as fed was about as 1:1.5. The results obtained showed that five of the six complete accountings of the calcium were positive or in equilibrium, while one was negative. They conclude that the calcium balance was favorably influenced by feeding tricalcium phosphate which was precipitated in a starch paste. They believe this influence may be due to the fineness of division of the tricalcium phosphate.

Telfer and Crichton (1924) found from a study of normal and modified goat's milk fed to kids from birth that the normal mineral content of milk is probably near the minimum requirement of the species. They found that the rate of ossification could be considerably increased by a greater concentration of mineral matter and that a small reduction is followed by defective growth and impaired nutrition.

Osteomalacia in Animals.—Van Sageghen (1919), in discussing osteomalacia of the equidae, speaks of it as osteoporosis. Whenever the disease occurred among horses or donkeys in the Belgian Congo the soil of the district was found to be very poor in calcium and phosphorus and little or no calcium was found in the water. No cases occurred in districts in which the water contained calcium.

Observations on Bone Disorders in Swine.—Zilva, Golding, Drummond and Coward (1921) attempted to produce rickets in pigs on a diet deficient in vitamin A. Pigs were fed on extracted dried milk, cream (cow's milk), purified casein and a salt mixture (McCollum, 185 mixture). Control animals were fed the same diet except that for the cream autoclaved olive oil was substituted. They stated "The results of our experiments offer no definite information on the relation of the fat-soluble factor to the etiology of rickets. We are, however, of the opinion that if the deficiency of this factor alone bore the same relation to the etiology of rickets as that of the anti-scorbutic and anti-neuritic factors to the etiology of scurvy and beri-beri, we should have obtained a better differentiation in the rachitic condition in the two groups." It seems probable that their experiments were made inconclusive through the inclusion in their diets of

the salt mixture 185, which contains both calcium and phosphorus. It is not possible to produce rickets in rats when this salt mixture is fed, even though the diet is deficient in vitamins A and D, since the calcium and phosphorus are present in amounts and proportions which promote calcification.

Elliott, Crichton and Orr (1922) fed pigs on diets of bran and oatmeal, together with an abundance of vitamins A, B and C, and state that the pigs developed rickets in 3 months. Another series of pigs were fed the same foods and under the same conditions but were supplied with calcium salts. These did not develop rickets. When the animals were fed a diet containing a minimum of vitamin A, but an adequate amount of calcium salt, the rickets syndrome did not appear, although the condition of the animals was not so good as that of the controls.

Husband, Godden and Richards (1923) discussed the influence of cod liver oil, linseed oil and olive oil on the assimilation of calcium and phosphorus in the growing pig. They stated that when the animals were in negative balance cod liver oil and olive oil each rapidly converted them into positive balances, whereas linseed oil was much slower in its action. They state that the diet which they used contained calcium oxid 0.393 and phosphorus pentoxid 3.712 per cent respectively. All experience of other investigators is contrary to theirs with olive oil and linseed oil.

Bohstedt and co-workers (1924) discuss the problem of rickets in swine as affected by nutrition. They state that lameness, unthriftness, partial paralysis or rickets in pigs is very prevalent, especially in winter and early spring. These abnormalities in the life of a pig may be symptoms of one and the same disorder. It has been demonstrated that deficient rations will cause an ailment which affects not only the bones but also other tissues of the body, especially nervous tissues. Apparently, they state, there is something lacking in winter rations which is usually contained in the summer ration, when rickets or lameness rarely occurs. Pasturing pigs has been found a preventive.

Grains or seeds and their by-products used so generally in winter rations of hogs do not furnish enough nor the right kinds of minerals and vitamins. It is apparently due to these two deficiencies that rickets occurs. They found that the addition of lime (calcium carbonate), phosphate as di-sodium phosphate or bone flour, without the addition of vitamins did not prevent rickets.

Bohstedt reports that the bones in pigs fed a diet very low in minerals but containing cod liver oil could be easily broken by a mere twist of the hand. Many spontaneous fractures occurred in pigs fed the basal ration and cod liver oil alone. Feeding both cod liver oil and bone flour produced the most uniform and economical gains. These results confirm those which McCollum, Simmonds, Shipley and Park obtained with rats, viz., the addition of vitamins carried by cod liver oil furnished a strong growth impulse but in the absence of a satisfactory mineral supply made brittle bones which were easily broken. We observed this condition with regularity in rats fed diets deficient in phosphorus and calcium, but containing cod liver oil.

Leg Weakness in Pigs.—Maynard (1922) reported studies on leg weakness or posterior paralysis in pigs. Four pigs at the age of 12 weeks were put in a dry lot and were fed for 90 days the following diet: 1 part yellow hominy; 1.4 parts of pasteurized skim milk, together with a salt mixture of charcoal, ground limestone and sodium chlorid. At the end of 90 days 3 of the pigs had developed a stiffness of the hind legs and were in a weakened condition. The skin was dry, scaly and contained livid spots. One of the animals had a very swollen knee. The stiffness increased and was accompanied by great pain. Two of the animals developed paralysis in the hind legs and could not stand. There was rapid loss of weight. The mineral mixture was then replaced by precipitated bone meal, precipitated calcium carbonate and sodium chlorid, and four ounces of carrots were given daily. At the end of 5 weeks the stiffness had disappeared and the skin was nearly normal. They had completely recovered at the end of 7 weeks.

Maynard and co-workers (1924) state that the essential lesions in the posterior paralysis of pigs are an osteoid rather than an osseous tissue formation at the epiphysis and costochondral junctions. These indicate a lessened amount of calcification somewhat suggestive of rickets without the usual deformities found in this condition. The condition is not due to a deficiency of calcium and phosphorus in the diet, since these were supplied. It seems to be due to a lessened assimilation of these materials. Additions of either alfalfa or cod liver oil produce recovery of the cases already affected. The symptoms in the early stages and the lesions in the paranchymatous organs are similar to those that are supposed to be diagnostic of hog cholera.

Calcium Metabolism of Laying Hen.—Wheeler (1919)

found that rations deficient in calcium but carrying an abundance of magnesium produced a noticeable shortage of calcium and of mineral matter in the bones of fowls and ducks. In no instance was magnesium found to serve to any extent in place of calcium for such purposes as egg shell material when calcium was lacking. Strontium was found capable of replacing calcium to a considerable extent in the egg shell and in the bones. Fewer eggs were obtained from hens fed added magnesium or strontium salts than those fed calcium salts.

Buckner, Martin and Peter (1924) reported experiments on the calcium metabolism of the laying hen. They found that hens receiving limestone produced larger eggs than those which did not receive it. The hens receiving corn, buttermilk and limestone produced larger eggs than those receiving corn and a mash containing tankage and limestone, both the shell and liquid part being heavier. They found calcium carbonate greatly increased the efficiency of buttermilk for egg production. The hens getting buttermilk and corn were at all times in a notably better condition than those getting corn and a mash containing tankage without the buttermilk. They state that the practical conclusion for the poultryman is that for satisfactory egg production laying hens on the above diets must be given additional calcium, which should be in the form of the carbonate.

Leg Weakness in Chicks.—Hart, Halpin and Steenbock (1921) reported that green cabbage, green clover or orange juice when fed with a synthetic diet in the absence of sufficient roughage did not prevent leg weakness in chickens. They found 10 per cent of paper to be a very good roughage for chicks. With the use of paper and a synthetic diet chicks made good growth. They determined that leg weakness is not scurvy in chickens. They (1922) reported a further study of leg weakness in baby chicks. Young chicks were reared under confinement to 800 gm. or more in weight on a diet consisting of white corn, skim milk, a salt mixture and cod liver oil. Wood shavings were provided for litter. The experiment was continued during 11 weeks, which they state is beyond the critical time for the development of leg weakness. They believe that this species requires a liberal supply of the vitamins in cod liver oil during the growing period. Vitamins B and C did not appear to be so necessary, although under certain conditions the requirements for vitamin B are very high. When cod liver oil was omitted from the diet the birds died in from 4 to 6 weeks with symptoms which were described

FIG. 24.—A contrast between the effect of green plant tissue and radiant energy. Both chicks received a synthetic ration composed of alcohol-extracted casein 18, dextrin 60, salt mixture 5, agar-agar 2, yeast 15, and 5 per cent of fresh green clover calculated on the basis of the dry weight of the clover.

	Left	Right
Initial weight, June 19, 1923	35 grm.	38 grm.
Weight July 10, 1923	77 grm.	95 grm.

July 12, 1923, began exposure of one chick (right), to sunlight one-half hour daily, both birds being continued on the same ration.

	Left	Right
Weight August 8, 1923	95 grm.	235 grm.

(Courtesy of Professor E. B. Hart, Wis. Agr. Exp. Sta.)

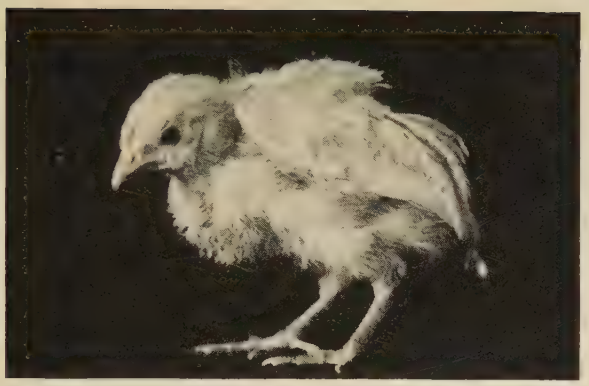


FIG. 25.—The effect of limited radiant energy (sunlight) and a rachitic ration. This bird is one from a group fed white corn 97, calcium carbonate 2, sodium chloride 1, and skimmed milk, ad libitum. Sunlight was limited to ten minutes per day. Weight 140 grams at six weeks of age. It is perfectly evident that the specimen is an abnormal one. (Courtesy of Professor E. B. Hart, Wis. Agr. Exp. Sta.)

FIG. 26.—The effect of unlimited radiant energy (sunlight) and a rachitic ration. This bird was taken from a group that received white corn 97, calcium carbonate 2, sodium chloride 1, and skimmed milk ad libitum. This group was exposed to sunlight the entire day. Weight 210 grams at six weeks of age. The group at this age appeared normal although vitamin A was in low amounts in the ration. (Courtesy of Professor E. B. Hart, Wis. Agr. Exp. Sta.)

by animal husbandrymen as leg weakness. The inorganic phosphorus content of the blood was low in these birds as compared with those receiving cod liver oil.

Mussehl and Calvin (1921) presented data which show that the application of the biological method for the analysis of a food-stuff to corn, using the chick as an experimental subject, yields results entirely comparable with those secured with the rat or swine.

Emmet and Peacock (1922) employed rat rations modified by increasing the concentration of protein and including roughage, as charcoal, oyster shell or grits, in chick feeding experiments. The chicks were 3 to 4 weeks old. The diets were deficient in vitamins A and B. Chicks receiving diets deficient in vitamin B developed polyneuritis in 10 to 12 days. Those receiving diets deficient in vitamin A lived 14 to 21 days. The latter developed leg weakness, poor plumage, edema about the eyes, which is a condition resembling ophthalmia, also beading of the ribs, atrophy of the testes and hypertrophy of the adrenals and gall bladder. They believed the requirements of the chick to be less for vitamin C than for A and B. Plimmer and Rosedale (1923) have demonstrated that the chick does not require vitamin C.

Effect of Sunlight on Development of Chicks.—Hart, Steenbock, Lepkovsky and Halpin (1923) studied the effect of exposure to direct sunlight on the growth of chicks. They found that one-half hour's exposure to direct sunlight was more potent in furnishing the anti-rachitic equivalent than was 5 per cent of the synthetic ration fed as green clover calculated on a dry basis. They (1924) fed two groups of chicks on the following diet: Corn 97.0, NaCl 1.0, $CaCO_3$ 2.0 per cent, and skim milk ad libitum. One group was fed white corn, the other group yellow corn. Both groups were exposed to sunlight daily. Those birds receiving the white corn which was low in the vitamin A did not respond to the sunlight treatment, whereas those receiving yellow corn and sunlight grew to be beautiful birds.

Rickets in Chicks.—Hughes (1924) found a condition to develop in chicks identical with rickets in mammals when the birds were given satisfactory food but received sunlight filtered through glass. Chicks receiving the same food and treatment but exposed to direct sunlight for a few hours daily developed normally. He found ultra-violet light to have the same beneficial effect as direct sunlight. He confirmed the work of Hart, Halpin and Steenbock that cod liver oil prevents leg weakness

in chicks. Hughes also (1924) found that 0.5 per cent of cod liver oil was effective in preventing leg weakness, but 10 per cent of either sprouted oats or green alfalfa was not sufficient to prevent the condition. He observed that ultra-violet light had a marked beneficial effect on the well-being of the chicks as shown by an increase in the number of eggs produced, their chemical composition and their hatchability.

Dunn (1924) reported experiments on chicks which indicated that cod liver oil which had been preserved in a dry mixture containing 85 per cent of starch and 15 per cent of oil, which was corked in bottles and stored in the dark for 6 months, had lost its anti-rachitic properties as compared with raw cod liver oil which had been stored for the same time and under the same conditions. He noted no symptoms of vitamin A deficiency in the chicks receiving the stored oil but stated that the evidence of deficiency of the anti-rachitic factor was unmistakable.

CHAPTER XXII

THE NURSING MOTHER AS A FACTOR OF SAFETY IN THE NUTRITION OF THE SUCKLING

Diet During Pregnancy and Lactation.—The period of pregnancy and of lactation are of special significance in the lives of both mother and offspring. No one would contend that all children are born with equal vitality. Those researches relating to the effects of experimental diets on the pregnant and the nursing mother are, therefore, of special interest, as are also those observations which show the effects on the mother and child under the different conditions of life where marked contrasts in the quality of the food supply prevailed. These topics will form the subject of the present chapter.

Since this book deals with principles rather than with practical dietetics, the management of the diet of the pregnant mother will not be considered in detail. Since it is of the greatest importance that the expectant mother should be under the supervision of her physician, it is best to leave the planning of her dietary to him. Day (1923) and Mendenhall (1924) have discussed in detail protective feeding during pregnancy.

Osteomalacia in Women.—The observation of Maxwell (see p. 367) on the widespread occurrence of osteomalacia among women in Northern China and Southern Manchuria already described illustrates how the safeguarding of the calcium metabolism during pregnancy may become a problem of the first order in public health work. The not infrequent occurrence of rickets during the nursing period emphasizes the fact, not generally appreciated, that breast feeding has its limitations in safeguarding the infant's health, and that proper feeding of the mother at this time is as important as breast feeding itself.

Newborn Young Dependent on Milk.—For a time after birth every mammal is incapable of taking the type of diet which suffices for the nutrition of the adult. It must have milk for a certain period, although the duration of dependency is not the same for all species. The guinea pig appears to have the shortest

period of dependence upon the mother. It is born in a very advanced state of maturity and can eat grass or succulent vegetables during the first or second day of postnatal life. The young rat is born in a very immature state; it does not open its eyes until the fourteenth to the sixteenth day of life. It may be weaned at the age of 25 days provided the diet of the mother during nursing is satisfactory. If the mother's diet has been faulty in any respect it may be necessary to prolong the nursing period to 50 days during which time the young supplement their milk diet with the diet of the mother. The young pig (swine) when six to eight weeks of age eats fairly liberally of forage plants, cereals or roots.

The human infant must live during the first year of life on a diet consisting in great measure of milk. It cannot thrive in an optimal manner without a fairly liberal supply of milk during the early years of childhood. It seems certain that a common error in America at the present time is too great a substitution of other foods for milk during the first years in the lives of children.

Milk Secretion as Related to Diet.—One of the problems in which we have been interested for several years is the relation of diet to milk secretion in the rat. Babcock (1905) described experiments with cows which he deprived of sodium chloride, other than that contained in their food, for 15 months. He noted no decrease in the milk yield until they began to fail rapidly. The fat content of the milk from cows with an inadequate salt supply was slightly higher than in the control group. Eccles and Palmer (1916) reported very interesting experiments on the composition and yield of milk in cows fed superabundant rations. They also reported on the influence of under-feeding. They state that their data relating to the effect of the plane of nutrition on the milk flow indicate that secretion of milk is controlled by at least two factors. These may be designated (a) chemical, and (b) nervous. The chemical stimulus is the predominating one immediately following parturition. It is this stimulus that fixes the maximum flow for each individual animal. Just what controls this stimulus they are unable to say but they believe it to be a problem of heredity as well as of physiology.

Their experiments with super-feeding showed very conclusively that cows are unable to increase their milk flow beyond the fixed stimulus for this function in the individual. A high class dairy cow is one born with a strong inherent tendency to secrete milk,

and the general problem in feeding is to supply the cow with sufficient nutrients to support the milk production which she has inherited. There were several factors which influenced the effects of underfeeding, viz., the stage of lactation, the degree of underfeeding, the character of the ration, the state of flesh of the animal, the plane of nutrition of the animal previous to under-feeding and the length of under-feeding.

Meigs (1922) has given an excellent review of the early studies of animal husbandry investigations relative to the effect on the quality of the milk as induced by changing the various components of the diet. He points out that many of the experiments were poorly planned and controlled but there is nevertheless an agreement that the quantity of the milk yielded is highly dependent on the quantity of the protein supplied in the food.

Faulty Diet and Milk Production.—Since many laboratory experiments had demonstrated that young rats could not synthesize the vitamins A and B, but must derive them from their diet before growth could take place, the question arose as to whether or not the mammary gland could synthesize these essentials for the nursing young when the diet of the mother did not contain them. In order to discover whether the mammary gland could synthesize vitamins A and B, McCollum and Simmonds (1916, 1918) carried out a series of experiments with lactating rats whose diets were faulty in known respects. The rats were kept on a diet of natural food-stuffs until the young were born. After the young were delivered the litters were reduced to four in order that the nutritive undertaking of the mother should be in no case burdensome. Several series of experiments were conducted in which the basal ration consisted of purified casein, dextrin, inorganic salts, and agar-agar. This ration is adequate for growth in the rat except for the absence of vitamins A and B. In a second series of experiments the same ration was used except that butter fat was added to furnish vitamin A; a third series received the vitamin B in the form of an alcoholic extract of wheat germ, but no vitamin A was added to the diet. The control group had both the vitamins present in the diet in the form of an alcoholic extract of wheat germ and butter fat. The results of these experiments showed very conclusively that the vitamins A and B pass into the mother's milk only when they are present in the diet of the mother.

We carried out another series of experiments with nursing rats to see to what extent the mother could supplement in milk syn-

thesis a very inadequate diet through the agency of the mammary gland. The animals were kept on an adequate diet until they delivered their young. The litters were reduced to four young. Diets faulty in known respects but derived from natural foods were given the nursing mothers instead of the purified diets discussed above. The experiments demonstrated the following facts: (a) that the mother rat could take a single seed as wheat, corn or rolled oats, and produce milk in sufficient quantity and of such quality that growth could proceed at a subnormal rate, whereas the young could not themselves grow at all on the mother's diet; (b) when the inorganic deficiencies of the seed are corrected the mother can induce much greater growth in the young than without such correction (see rat 1019, Chart IX); (c) the improvement of the protein or vitamin A content of the seed causes little improvement in the quality of the milk when the mineral content of the diet remains uncorrected. It had been abundantly demonstrated that young rats cannot grow on a diet of cereal grains and that the first limiting factor in such a diet is the mineral content. The nursing mother can take such a diet and put into the milk for a limited time an inorganic salt content which is very much more satisfactory for the nutrition of the young than is contained in the seeds composing her diet.

The question may be raised as to whether or not the rats were producing milk on these faulty diets. The observation of Decaisne (1871) that during the siege of Paris young and vigorous women were able to produce enough milk to maintain their infants, bears on this point. In some instances the mothers, while partially fasting, increased their infants' weight. Andrews (1912) attempted to throw light on the etiology of infantile beri-beri by inducing Filipino women whose infants had died of this disease to nurse young pups. All of these pups developed paralysis of the posterior extremities, which is one of the characteristic symptoms of the disease. Our experiments with rats confined to a diet free from vitamin B confirm and explain Andrews' results. They establish the fact that milk production can take place under dietary conditions such that the product is deficient in certain complexes necessary for growth. Dr. E. Roxas of the Philippine Islands and Dr. A. do Amaral of Brazil (personal communication) inform us that mothers suffering from beri-beri produced considerable quantities of milk. The work of Kennedy and Dutcher (1922) also confirms the observation that the diet of the animal determines the amount of vitamins A and B in the milk.

Kennedy, Palmer and Schlutz (1923) found breast milks to vary greatly in their content of vitamin B. Ten c.c. of a breast milk as a rule was not so potent as a source of this vitamin as 10 c.c. of cow's milk. Their results indicate that the vitamin A content of human milks is as a rule more satisfactory than the vitamin B content. The milk of a mother whose diet was deficient in vitamins A and B produced milk which was deficient in these when it was tested on rats.

Nelson, Lamb and Hellar (1922) demonstrated that young rabbits nursing a mother rabbit which was being fed a diet deficient in vitamin A developed xerophthalmia. The eyes of the nursing young became clear and bright when butter fat was added to the mother's diet. Hart, Steenbock and Ellis (1920) demonstrated that the milk of cows which had been confined for a year or more to rations consisting of air-dried grains and forage plants and which had never been allowed during these months to eat any fresh or green food was very deficient in vitamin C. Their results show clearly that the content of the anti-scorbutic substance in milk is dependent upon the diet.

Nutritive Value of Winter and of Summer Milks.—Dutcher and co-workers (1920) have shown that 20 c.c. of summer milk were superior in anti-scorbutic potency to 60 c.c. of winter milk. Their results showed that when a cow passed from a green pasture to dry feed her milk did not immediately undergo deterioration in respect to its anti-scorbutic value. Only after a period of 5 to 8 weeks was there sufficient change in the quality of the milk to show its effects on guinea pigs. On ingesting fresh food the milk immediately rose in its anti-scorbutic value.

Diet and Composition of Eggs.—A remarkable observation has been made by Pollard and Carr (1924) which is of interest in connection with the possible variation in the composition of a secretory product of a gland. They fed pigeons on diets restricted to a single plant seed supplemented with grit, oyster shell, ground bone, salt, charcoal and tap water. The birds were therefore restricted to the seed grain for protein and vitamins. They employed wheat, corn, oats, barley, rye, kafir, hemp, popcorn, sunflower seed, soy bean seed, field peas and buckwheat. The birds were fed about 6 months before the samples of eggs were secured for analysis. The following table shows the remarkable differences in the chemical composition of the eggs secured from birds on the different diets.

TABLE XXI

THE NITROGEN DISTRIBUTION OF PIGEON EGGS FROM VARIOUS CEREAL GRAINS EXPRESSED IN PER CENT OF THE TOTAL NITROGEN OF THE EGGS

Ration	*Per cent of N*	*Ammonia N*	*Melanin N*	*Amino N in bases*	*Non-amino N in bases*	*Amino N in filtrate of bases*	*Non-amino N in filtrate of bases*	*Total summation*
Rye	1.39	0.00	4.06	12.66	9.92	58.84	14.12	99.60
Rye	1.25	0.00	4.00	12.20	9.70	60.00	13.10	99.00
Kafir	1.54	1.76	1.92	14.12	22.48	53.94	4.20	98.42
Kafir	1.66	1.80	1.98	14.32	22.16	53.28	5.16	98.70
Wheat	1.42	0.71	3.90	19.16	13.00	60.44	0.72	97.93
Wheat	1.48	0.81	4.20	18.60	13.52	62.04	1.38	98.55
Oat	1.28	0.18	3.22	12.06	9.80	71.40	2.00	98.66
Oat	1.20	0.20	3.32	12.34	9.80	70.60	2.74	99.00
Hemp	1.43	2.11	1.00	11.74	9.54	67.40	7.10	97.78
Hemp	1.53	2.00	1.00	11.84	9.58	66.80	7.80	99.02
Canada field peas	1.24	0.00	1.33	11.00	18.97	.....	.....	.....
Canada field peas	1.36	0.00	1.50	10.40	18.93	.....	.....	.....
Barley	1.39	2.07	0.66	14.74	17.57	50.00	1.94	98.00
Barley	1.47	1.72	0.80	14.34	18.93	58.00	2.94	97.73
Corn	1.26	0.91	3.47	11.26	12.22	70.00	3.20	101.06
Corn	1.38	1.00	3.52	11.00	12.10	70.40	2.48	100.50

It will be seen from the table that there is a wide difference in the per cent of total nitrogen in the different lots of eggs, the highest, kafir, containing 1.6% and the lowest, oats, 1.24%. The nitrogen distribution or character of proteins was found to be quite different in the various lots. No ammonia nitrogen was found in the rye or field pea eggs, whereas those from kafir, hemp and barley contained nearly 2 per cent. It was only the eggs which contained a high melanin (tryptophan) content which were hatchable. It will be noted that when pigeons were called upon to decide which single grains are the most satisfactory for maintenance and egg production, their answer seems to rate the grains in the following order of decreasing efficiency: wheat, rye, oats, corn, kafir, barley, field peas, hemp and soy beans, popcorn, buckwheat and sunflower. The last three were insufficient for maintenance of body weight and no eggs were produced from these lots. The kafir is comparable to rye in its egg producing properties but the eggs were abnormal in composition and none of them were hatchable. There were usually four eggs laid in place of two when kafir was fed.

The secretion of the material of the yolk and of the white by the ovary and oviduct respectively represent the elaboration of a highly organized system designed by nature for the development of a young bird. One would expect, from knowledge hitherto available, that if the entire list of substances necessary for the formation of the proteins and other complexes was not satisfactory, the bird would fail to lay eggs. It seems certain, from the above data, that eggs were produced which contained proteins of abnormal structure, and that a protein structure departing widely from the normal pattern was formed. In the light of such data it seems not improbable that the composition of milk proteins may differ to a very significant degree from the normal when the proteins of the lactating animal are peculiarly constituted.

Diet and Quality of Milk Produced.—We have studied the effect on the rate of growth of young rats on diets of different types when fed to the lactating mother. The results are shown in Tables XXII, XXIV and XXVI. In Table XXVI are shown the weights of litters of young containing different numbers and at various ages during the nursing period, as well as later after they became able to supplement the mother's milk with her diet. These weights serve as controls for comparison of the weights of the young of other mothers whose diets were faulty in

(Continued on page 464.)

TABLE XXII

SHOWING THE RATE OF GROWTH OF YOUNG RATS WHEN THE DIET OF THE MOTHER IS OF FAIRLY SATISFACTORY TO GOOD QUALITY.

No. of Experiment.	Wt. of Mother After Young Were Born. Grams.	Wt. of Mother 10 to 19 Days Later. Grams.	Wt. of Mother at End of Nursing.		No. of Young.	Weight of Young at Different Ages.					
			Days	Grams.		Days.	Grams.	Days.	Grams.	Days.	Grams.
1236.....	142	... ...	40	146	7	16	133	30	286	40	544
2811.....	205	19th day... 225	28	215	10	19	225	28	387	..	...
2069*.....	215	10th day... 232	22	205	4	10	100	22	197	..	...
2767.....	270	10th day... 275	29	270	6	19	163	29	330	..	...
2153*.....	175	15th day... 182	28	192	5	15	137	28	277	..	...
2436.....	200	... ...	32	185	8	18	188	32	420	..	...

* The diets of lots 2069 and 2153 were less satisfactory than the others. On these two diets the animals in the second and succeeding generations became progressively stunted.

TABLE XXIII

Composition of Diets Used in Experiments Described in Table XXII.

No. of Experiment.	Beefsteak Grams.	Wheat Grams	Maize Grams.	Rice Grams.	Oats Grams.	Peas Grams.	Navy Beans Grams.	Casein Grams.	Dextrin Grams.	Milk Powder* Grams.	Butter Fat Grams.	Wheat Flour† Grams.	Corn Meal‡ Grams.	Cabbage§ Grams.	$CaCO_3$ Grams.	NaCl. Grams.	KCl Grams.	Cod Liver Oil Grams.
1236..	50.0								43.5		3.0				1.5	1.0	1.0	
2811..		25.0	11.5	9.5	9.5	9.5	9.5	10.0		10.0	3.0				1.5	1.0		
2069..	10.0			8.0	8.0	8.0	8.0					30.0	15.5	10.0	1.5	1.0		
2767..		30.0	19.0	9.5	9.5	9.5	9.5	10.0							0.5	1.0		1.0
2153..	10.0			9.5	9.5	9.5	9.5					30.0	19.5		1.5	1.0		
2436..	10.0	30.0	19.0	9.5	8.5	8.5	8.5				3.0				1.5	1.0		

* Merrill-Soule Whole milk powder.
† Bolted wheat flour.
‡ Degerminated cornmeal.
§ Desiccated cabbage, subsequently steamed and dried.

TABLE XXIV

SHOWING THE STUNTING EFFECT ON THE NURSING YOUNG OF DEFICIENCY OF CALCIUM IN THE DIET OF THE MOTHER.

No. of Experiment.	*Wt. of Mother After Birth of Young. Grams.*	*Wt. of Mother 10 to 20 Days Later.*		*Wt. of Mother at End of Nursing.*		*No. of Young.*	*Weight of Young at Different Ages.*					
		Days.	*Grams.*	*Days.*	*Grams.*		*Days.*	*Grams.*	*Days.*	*Grams.*	*Days.*	*Grams.*
2785 *	165	20	157	35	165	3	20	73	35	162	..	...
2733 †	150	13	150	34	151	4	13	48	34	151	61	184
2069 ‡	215	10	232	22	205	4	10	100	22	197	..	...
2732 §	155	18	160	45	150	6	18	90	34	210	54	247 (Five young)
2767 ‖	270	19	275	29	270	6	19	163	29	330	..	...
2782 ¶	182	14	160	30		8	19	109	30	172	39	210
2436 **	200	..	...	32	185	8	18	188	32	420	..	..

* Diet consisted of wheat, maize, rice, oats, peas, beans, casein, NaCl, butter fat and cod liver oil. (See p. 461, etc.)

† 2733 Diet same as 2785 except that cod liver oil was the only fat added, and casein was substituted by beefsteak.

‡ Diet consisted of beefsteak, oats, peas, beans, rice, cabbage, wheat flour, corn meal, $CaCO_3$, and NaCl.

§ Diet similar to 2733 but with less cod liver oil. (See p. 461.)

‖ Diet closely similar to 2785, but contained 0.5 per cent of added $CaCO_3$. Note benefit to growth of young.

¶ Diet like 2785 except that casein was omitted. (See p. 461.)

** Diet similar in properties to the others used in these experiments but with the optimal amount of calcium carbonate added (1.5 per cent). Note the remarkable stimulating effect on the growth of the young.

TABLE XXV

COMPOSITION OF DIETS USED IN EXPERIMENTS DESCRIBED IN TABLE XXIV.

No. of Experiment.	Beefsteak Grams.	Wheat Grams.	Maize Grams.	Oats Grams.	Peas Grams.	Navy Beans Grams.	Rice Grams.	Casein Grams.	Cabbage * Grams.	Wheat Flour † Grams.	Corn Meal ‡ Grams.	Butter Fat Grams.	Cod Liver Oil Grams.	$CaCO_3$ Grams.	NaCl Grams.	$NaHCO_3$ Grams.
2785		25.0	20.0	10.0	9.5	9.5	10 0	10.0				2.0	1.0		3.0	
2733	10.0	30.0	19.5	8.5	8.5	8.5	9.5						3.0		1.0	1.5
2069	10.0			8.0	8.0	8.0	8.0		10.0	30.0	15.5			1.5	1.0	
2732	10.0	30.0	19.5	9.5	9.5	8.5	9.5						1.0		1.0	1.5
2767		30.0	19.5	9.5	9.5	9.5	9.5	10.0					1.0	0.5	1.0	
2782		30.0	20.0	11.0	11.0	11.0	11.0					2.0	1.0		3.0	
2436	10.0	30.0	19.5	8.5	8.5	8.5	9.5					3.0		1.5	1.0	

* Cabbage desiccated, steamed and subsequently dried.
† Bolted wheat flour.
‡ Degerminated corn meal.
§ Norwegian cod liver oil employed in all experiments.

TABLE XXVI

Showing Weight Changes of Female Rats and Their Young During the Nursing Periods as Influenced by the Character of Their Diet.

No. of Experiment.	Weight of Mother After Young Were Born. Grams.	Weight of Mother 10 to 18 Days Later.		Weight of Mother When Through Nursing.		Weight of Young at Different Ages.						No. of Young.	Generation on Diet.
		Days.	Grams.	Days.	Grams.	Days.	Grams.	Days.	Grams.	Days.	Grams.		
2436 *.....	200	17	250	28	222	17	140	28	258	..	...	6	1st
"	257	11	277	25	265	11	185	25	200	..	...	4	2nd
"	182	..	...	30	180	16	132	30	305	..	...	6	3d
"	180	17	195	30	195	17	170	30	335	..	...	6	4th
2153 †.....	220	..	...	31	225	11	170	31	400	..	...	10	1st
"	175	15	182	28	192	15	137	23	208	28	277	5	1st
2069 ‡.....	200	27	172	35	177	12	115	27	243	35	342	7	1st
"	167	31	173	38	175	17	135	31	275	38	390	6	2nd
"	135	14	150	28	145	14	90	28	202	..	...	4	3d
2360 §.....	175	31	175	..	...	12	95	19	148	31	265	6	1st
"	142	22	152	33	154	14	120	27	225	33	350	7	2nd
"	115	30	128	37	132	..	...	30	209	37	262	7	3d

* Diet very good. It consisted of wheat, maize, oats, rice, peas, beans, beefsteak, NaCl, $CaCO_3$, and butter fat. Full formula is given on page 463. There was no deterioration observable in the 4th generation.

† The females of the 2nd generation never had any young. The diet consisted of milled wheat flour and maize meal, polished rice, oats, peas, navy beans, beefsteak, NaCl, and $CaCO_3$. The first generation did very well for about eight months, then failed rapidly. The 2nd generation failed very early without reproducing. Formula on page 463.

‡ Similar to 2153, but contained 10 per cent of cabbage. Family at first did well, but deteriorated rapidly with each succeeding generation. The 4th generation were in poor condition and had no young.

§ Similar to 2436, but contained no butter fat, and was alkalinized with $HNaCO_3$. Fertility good and infant mortality low, but each succeeding generation was decidedly smaller than its parents. The fourth generation was discarded in a stunted condition at the age of 2½ months. These experiments show the value of continuing observations over several generations, for they illustrate the tendency toward degeneration when the diet is faulty in even a slight degree.

TABLE XXVII

COMPOSITION OF DIETS USED IN EXPERIMENTS DESCRIBED IN TABLE XXVI.

No. of Experiment.	*Beefsteak Grams.*	*Wheat Grams.*	*Maize Grams.*	*Oats Grams.*	*Peas Grams.*	*Navy Beans Grams.*	*Wheat* Flour Grams.*	*Corn† Meal Grams.*	*Rice‡ Grams.*	*Cabbage§ Grams.*	*Butter Fat Grams.*	$CaCO_3$ *Grams.*	$NaHCO_3$ *Grams.*	NaCl *Grams.*
2436....	10.0	30.0	19.5	8.5	8.5	8.5			9.5		3.0	1.5		1.0
2153....	10.0			9.5	9.5	9.5	30.0	19.5	9.5			1.5		1.0
2069....	10.0			8.0	8.0	8.0	30.0	15.5	8.0	10.0		1.5		1.0
2360....	10.0	30.0	19.5	9.5	9.5	9.5			9.5			1.5	1.0	

* Bolted wheat flour.
† Degerminated corn meal.
‡ Polished rice.
§ Desiccated cabbage, subsequently steamed and dried.

some respect. Although the data are self-explanatory a few cases of marked contrast will be mentioned.

One of the females had 5 young on diet 2153 which is a satisfactory diet. At 15 days of age the collective weight of the young was 137 gm. A rat on diet 2365 (containing 9 per cent of protein, two-thirds derived from barley and one-third from navy beans) likewise had 5 young. When these young were 18 days of age they weighed collectively 70 gm. The protein moiety of this mother's diet was of rather poor quality and she was unable for this reason to secrete a satisfactory milk supply. The tables afford several other examples of a similar nature.

Diet and Infant Mortality.—We have discussed in Chapter VII the high mortality of the nursing young on low protein diets. One of the most interesting observations made in connection with our rickets studies was in regard to the condition of nursing females on low calcium diets. Rats on diets comparable to Lot 2785 presented a highly characteristic picture. Even though the animals did not present any abnormal appearance, as is frequently the case on low calcium diets, they were, nevertheless, in a state of great nutritional instability. Their vitality was very much below that of rats on highly satisfactory diets. Their weakness was shown in a highly spectacular manner when nursing their young. They were nervous and apprehensive and in walking extended their legs and held their bodies higher than is the case with normal animals. Their gait was unsteady and slightly tottering. When they were at rest they had difficulty in rising to their feet. This condition did not usually appear while the females were nursing their first litters, but after the second litter had been nursed for about 15 to 16 days the symptoms usually appeared. The skeletons of these rats were very fragile and could be removed from the cooked flesh without fragmenting only with difficulty. Many of the bones broke into small pieces as soon as they were touched.

The young did not develop in a normal manner on these low calcium diets even though the mother sacrificed much from her skeleton and tissues. They remained small and many of them developed the pot bellies which are so characteristic of poorly nourished little rats. The mothers did not kill the young which was so frequently the case on low protein diets.

Hartwell (1922) reported data on the effect of diet on mammary secretion. She attributes spasms in the nursing young to an

FIG. 27.—This illustration shows the great differences in the size, stage of development and vigor in young rats, which may result from faults in the character of the diets of the mothers which they are nursing. These three rats were the same age. The mother of the two little ones received a diet which was deficient in the quantity and quality of its proteins.

excess of protein in the diet of the lactating mother. Several investigators have reported this condition in nursing rats. It occurs most frequently when the diet is very low in vitamin B. She (1924) states that with diets rich in protein the lactating rat must have a greater supply of vitamin B than when she is on a lower protein diet. Simmonds (1924) has described numerous experiments where rats were fed as much as 67 per cent of protein but with the diet chemically complete in other respects in which the young were weaned in an excellent condition. In no instance was there a spasm observed in a young rat nursing a mother whose diet contained an excessive amount of protein but which was otherwise satisfactory.

Composition of Milk of Pellagrous Women.—Voegtlin and Harries (1920) stated that pellagra sometimes occurs in breast-fed infants. They made an investigation of the milk of pellagrous women and reported that: "The volume may be normal or reduced, depending somewhat on the general nutritional state and food consumption of the patient. Very severe cases often secrete only 100 to 300 c.c. or less of milk per day, whereas we have records of milder cases which yielded approximately one-half to one liter. Lactose, fat, protein nitrogen and total solids were found to fall within the normal limits, but considerably below the normal average. The total ash and the phosphate were normal. A slight reduction in the quantity of calcium, magnesium and potassium was noted, whereas chlorides and sodium were present in larger amounts. The character of the diet had no influence on the percentage composition of the milk, with the exception that a change from a vegetable to a mixed diet was accompanied by a marked increase in the total non-protein nitrogen. The conclusion to be drawn from these observations is that well-marked cases of pellagra yield a milk which, as far as its composition with respect to the known milk constituents is concerned, does not show a sufficient deviation from the normal to account for the disease in nursing infants."

Quality of Breast-Milk Dependent on Diet.—During the great war the state of malnutrition among the children of Europe caused great concern. Chemical investigations of the composition of milks produced by women whose babies were not thriving were reported by Mömm and Kraemer (1918-19). They found the fat content to be essentially normal. The observation of Kaupe (1918-19) on mothers' milk during the great war is especially interesting in this connection. He states: "The absolute

amount of breast milk should be sufficient to yield normal increases in weight, but such was not the case. If it cannot be the fat content of the mothers' milk which causes so little increase in the weight of the babies, and if it cannot be shown by us that our mothers give proportionately too little milk, and finally, if it is quite impossible to show that there is a serious lowering of the percentage of carbohydrate or protein, then we must admit that the mothers' milk is undergoing changes which we know nothing of." The young did not gain in weight as they should. The average volume of milk produced by these mothers during the eleventh to the thirteenth day after their infants were born was 440 c.c. Weeks or even months frequently passed before the children regained the weight which they lost immediately following birth. This was true even when there was no evidence of disease. Kaupe was unable to explain the nature of the changes which rendered the breast milk inadequate and was inclined to attribute to psychic influences the deterioration of its nutritive value. Owing to the war he was not acquainted with the experimental work in America which showed how greatly the diet of the mother may influence the nutritive value of her milk. It seems very probable that the vitamin content of the milk was the limiting factor for growth, but this could not be determined by a chemical analysis.

Importance of Protective Foods in Diet.—In a civilized country where food is abundant and people are prosperous, people tend to cater to the sense of taste, and in so doing frequently spoil their diet through an unwise selection of food; in countries where food is scarce and but few articles are available, they tend to regard food mainly as an agency to relieve suffering through allaying the pangs of hunger. In the latter case the diet is generally faulty because of its simplicity and the absence of an effective supplemental relation among the different articles eaten. The experience of Andrews is a case in point. Hess (1918) made the observation that the negro women in the Columbus Hill District of New York whose diets were derived almost exclusively from milled cereal products, tubers and muscle meats, failed to nourish their infants satisfactorily, as was shown by the almost universal prevalence of rickets among them.

The occasional occurrence of scurvy in breast-fed infants represents an extreme example of specific starvation of the mother for a single dietary factor and the resulting injury to her nursing infant. Physicians and nurses have repeatedly reported to us

their observations that many women while nursing infants take diets for days or weeks derived in great measure from tea and toast, sugar and other articles of food equally unfit for the elaboration of milk. The nursing mother should always take a diet which will insure a satisfactory composition of the milk which she is to secrete. This can best be realized by including in her menus liberal amounts of milk and of salads (the protective foods). The salad should be composed in great measure of fresh foods such as lettuce, cabbage, celery, tomatoes, etc., and a liberal supply of citrus fruits. A liberal use of leafy vegetables, such as spinach, beet greens, turnip greens, kale and brussels sprouts, will tend also to insure a calcium supply as well as aid in relieving constipation. It is not enough for a diet to yield a proper caloric value, furnish protein according to the long accepted standards and afford variety and palatability. The specific dietary properties of the foods which enter into the diet are of paramount importance and must be given consideration. When the milk supply is limited, as is the case in China and Japan, mothers frequently nurse their children four to five years. They gradually accustom the children to a diet to which it would be impossible to restrict a child at an age at which American children are receiving cow's milk.

This chapter on the pregnant and nursing mother strikes the keynote of this book. It affords proof of the existence of and points the way to a remedy for one of the greatest sources of human inefficiency and suffering. Since it touches the root of the evil effects of one of the most colossal errors of civilization this chapter serves to illustrate most vividly the far-reaching importance of scientific nutrition in preventive medicine. It is only within the last few years that the diet of the human nursing mother in its relation to the quality of her milk has received the attention which it deserves even by investigators. Until the science of nutrition forced upon us the importance of the selection of foods, it was taken for granted that whatever milk was formed by the mammary gland was of good quality. A study of the data makes it apparent how dependent the nursing mother is on the character of her diet as regards the quality of the milk which she will produce. When her diet contains proteins of high biological value, an abundance of the vitamins, inorganic elements in proper amounts and a source of energy in the form of carbohydrates and fats, she produces a milk which will induce optimum gains in the weight of her young. In just so far as her diet falls

short in containing these essentials will the quality of her milk be reduced. The nursing mother cannot, except in a very limited degree, put into her milk from her bodily reserves that which she does not receive in her food supply. It is, therefore, of the greatest importance that the pregnant and nursing mother have a highly satisfactory diet in order that there shall be no shortage of the dietary essentials in her milk supply.

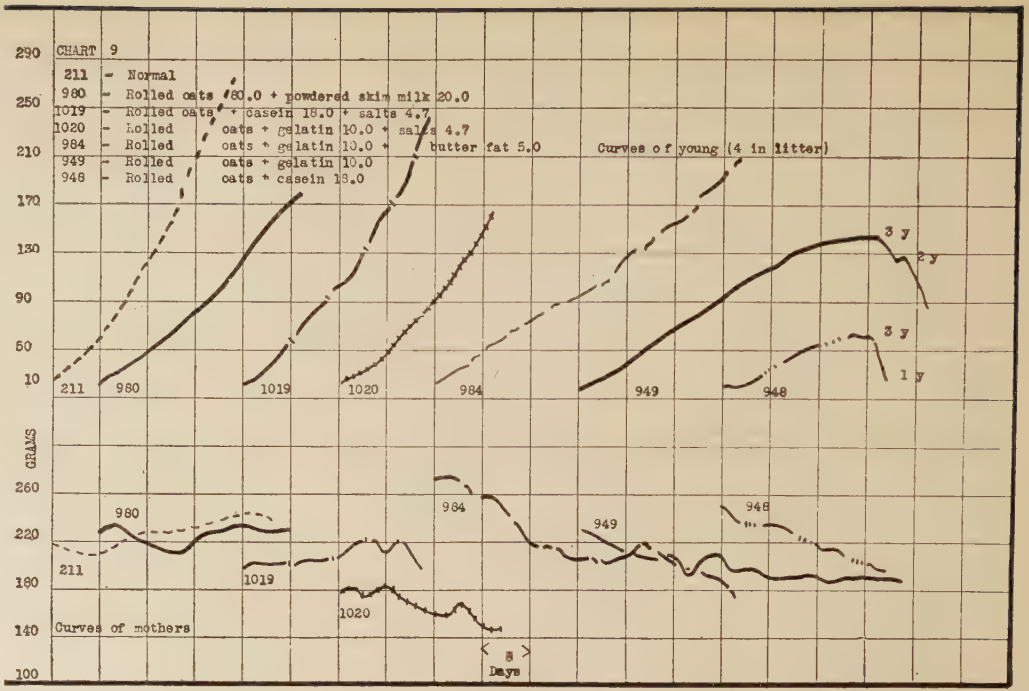


CHART 9
211 - Normal
980 - Rolled oats 80.0 + powdered skim milk 20.0
1019 - Rolled oats + casein 18.0 + salts 4.7
1020 - Rolled oats + gelatin 10.0 + salts 4.7
984 - Rolled oats + gelatin 10.0 + butter fat 5.0
949 - Rolled oats + gelatin 10.0
948 - Rolled oats + casein 18.0
Curves of young (4 in litter)
GRAMS
Curves of mothers
Days
3 y
2 y
1 y

CHART IX.—The curves at the top of this chart show the rate of growth of litters of four young each, when they were nursed by mothers taking the diets listed. No. 211 is an excellent diet containing a liberal amount of dried milk. On this the mother is able to induce rapid growth in her young.

Lots 980, 1019 and 1020 were given rolled oats and a protein supplement, either gelatin or casein, together with a mineral salt addition. Lots 984, 949, and 948 did not receive the supplementary salt additions. The inferiority of their young is contrasted with the first named group is very marked. Lot 984 was given a supplementary supply of vitamin A over that which was contained in the seeds. This prevented the early failure of the young as happened in Lots 948 and 949 which were not given an additional supply of this substance over what was furnished by rolled oats. These curves show clearly that the quality of the mother's milk is profoundly influenced by the character of her diet. If she is selectively fasted for mineral elements, vitamin A, vitamin B, or vitamin C, or if the proteins of her diet are not of good quality her milk is deficient in the substances which are wanting, relatively, in her diet. It is of the utmost importance that the diet of the nursing mother should be of very excellent quality with respect to all its parts.

The curves at the bottom of the chart are also of great interest. Note that those mothers which were fed rolled oats supplemented with a protein and a salt mixture were able to sustain their body weight much better than could those which were fed oats and a protein or oats, protein and butter fat. The draft upon the vitality of the mother while nursing young will be greatly relieved by making her diet highly satisfactory. Note that Lots 948, 949 and 984 lost weight much more rapidly than did those mothers whose diets were better constituted for the promotion of growth in their young.

CHAPTER XXIII

THE DIET IN RELATION TO THE TEETH

Prevention of Tooth Decay.—There is no subject of greater importance to human welfare than the preservation of the teeth. A score of ailments have been enumerated by medical writers believed to be the result of the introduction of bacteria or their poisonous products into the body through decayed teeth, or through diseased areas surrounding the necks of the teeth. There has been much discussion among dentists and others concerning the cause of tooth decay, but as yet there is no unanimity of opinion concerning it. Likewise the exact nature of the cause of pyorrhea is not clear. In caries the enamel becomes disintegrated and breaks down in a manner similar to what we should expect if acid were constantly applied to the surface of the tooth at one point. When a cavity is formed food particles and mouth secretions accumulate within it. It becomes the focus of active fermentation with further production of acid. Are the bacteria the primary cause of the carious cavity? Are there special kinds of bacteria which produce caries? Why do some persons escape caries while others develop it? Why is caries more frequent in early than in later life? Why are caries and pyorrhea seldom found in the same mouth? Are there differences in the structure of the teeth which make some immune and others susceptible to caries? What, if any, is the relation of the diet to tooth decay? Has the composition of the saliva anything to do with tooth decay? These are only a few of the questions which have been raised in recent years by those who are thinking critically about all the facts which observation has yielded, or who are experimenting in the field of preventive medicine.

Since bacteria are always demonstrable in the dentinal tubules in a carious cavity many have believed that caries is essentially a bacterial disease, and that the prevention of the disease lies in mouth hygiene, and that the preservation of the teeth is to be sought in cleanliness and the filling of cavities while they are small. The attitude of the U. S. Public Health Service may be

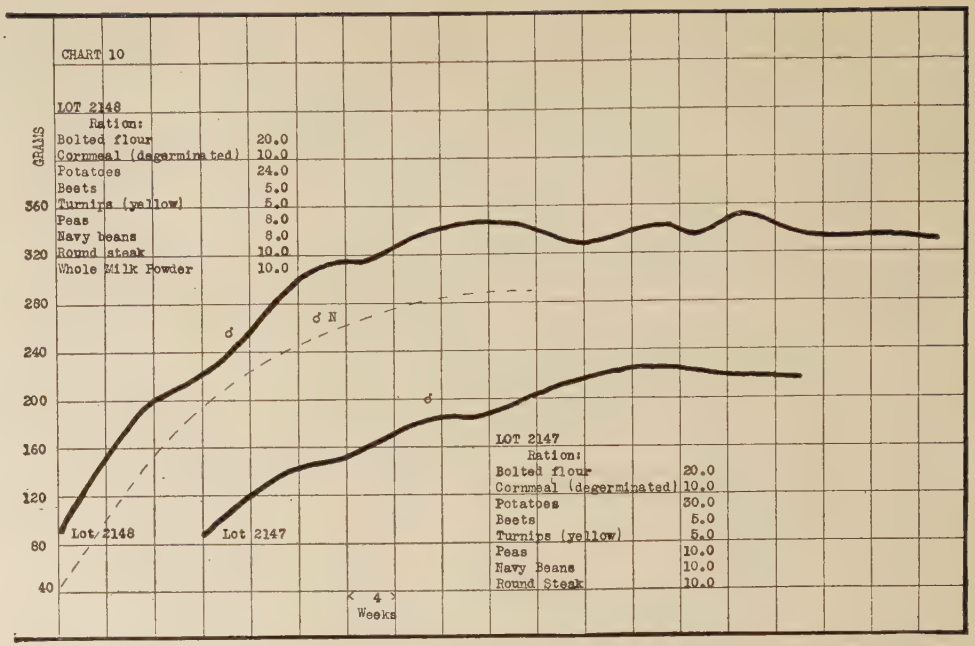


CHART X.—The two curves in this chart serve to illustrate the remarkable superiority of whole milk as a supplement to a mixture of cereal grains, legume seeds, tubers, and fleshy roots as compared with round steak, a muscle meat. Lot 2147 had a complex vegetable diet of the type described, supplemented with a liberal amount (10 per cent) of beefsteak. Their growth was slow and the animals never reached the full adult size. They also developed early in life the appearance of old age. Lot 2148 had essentially the same diet but supplemented with 10 per cent of whole milk powder. This served as a source of mineral salts and of vitamin A, in which diet 2147 was deficient, and made possible much more rapid growth, larger final size and much longer life. These curves illustrate the importance of the proper selection of foods, so as to be highly satisfactory, rather than the adherence to a diet which is somewhat faulty in one or more respects.

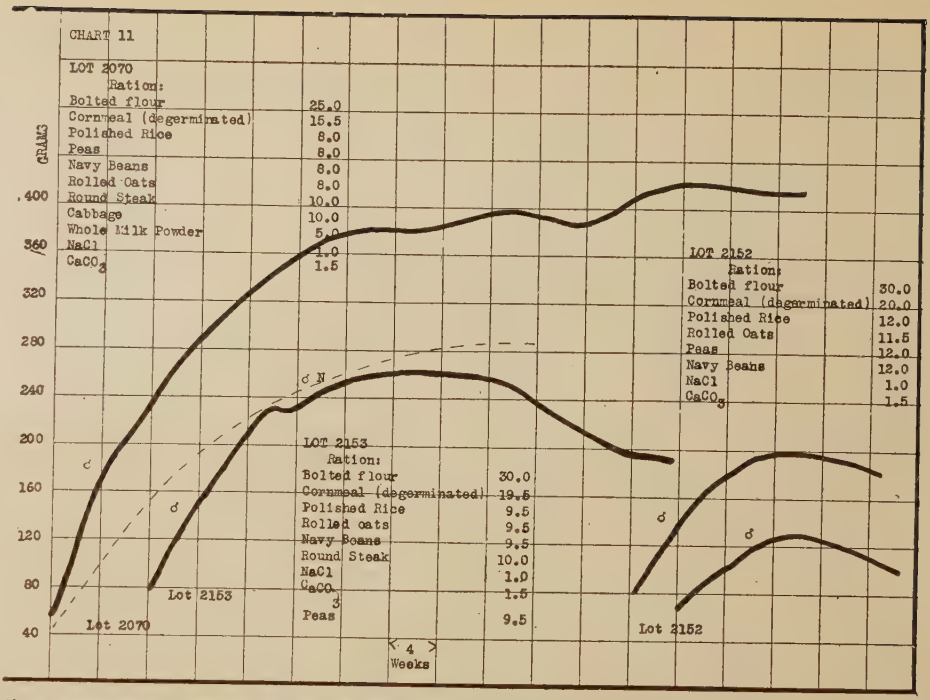


CHART XI.—The three curves shown illustrate the lack of vitamin A and the low quality of the proteins in a mixture of milled cereal products and legume seeds. In each experiment the chief mineral deficiencies were corrected by the addition of sodium chlorid and calcium carbonate. Lot 2152 had a diet which contained proteins of poor quality and was deficient in vitamin A. Note that the animals grew but little, and began very early to fail as shown by loss of weight. Their entire lives were not longer than the period during which a well-fed rat normally grows.

Lot 2153 had a diet essentially like that of Lot 2152 except that its proteins were enhanced by the inclusion of round steak. The diet was still deficient in vitamin A although by no means lacking in it. Growth was rapid for a time but the full adult size was never reached, and the animals early developed the characteristics of old age.

Lot 2070 had essentially the same diet as Lot 2153 except that cabbage and whole milk powder (Merrill-Soule) were included. These served to further enhance the proteins and to furnish vitamin A in moderate amount. The result is very spectacular. The animals grew rapidly to large size and maintained for a long period the characteristics of youth.

taken as representative of that of nearly all who have become active in promoting preventive dentistry. Clark and Butler (1920) stated that "Dental caries is caused by the action of bacteria or germs which normally inhabit the mouth. These germs, acting in the presence of food debris and certain elements in the saliva, result in the formation of an acid which attacks the enamel covering the exposed parts of the tooth, after which underlying softer parts become rapidly destroyed. Many other factors are actual and potential causes of dental decay and its progress, such as (1) low resistance of the teeth to decay because of developmental defect (antenatal and postnatal); (2) faulty diet (both of the mother during pregnancy and of the child); (3) neglect of dental attention through ignorance of the parents; (4) the cost of dental attention, a serious consideration with families of low economic status; (5) failure of the child to call attention to the condition of the teeth, either because it is too young or because of fear; (6) lack of dental facilities, so common in rural sections." It is pointed out by these authors that a very high percentage of undernourished children show marked evidence of dental decay. They cite the results of the examination of 270 children by the Public Health Service, which showed that 33 per cent of these had from 1 to 4 cavities, 48 per cent had from 4 to 8, and some had 9, 10 and 11 cavities. This excellent paper fails to emphasize the importance of including in the campaign for the preservation of the teeth an effort to change the character of the nation's diet, in order that developmental defects shall grow less common and less severe as time passes. Emphasis is laid entirely upon the importance of the dental hygienists, who limit their work to cleaning and polishing all surfaces of the teeth above the gum margins, to the dental clinic for the repair of the teeth already in a state of decay and to the teaching of mouth hygiene. Such a program is based upon the belief that cleanliness of the oral cavity is of first importance as a preventive measure for preserving the teeth.

Certain facts have been brought to light by experiments on animals which cast doubt on whether the above quotation takes into account all the essentials of an effective program of preventive dentistry. It does not mention any possible means of preventing caries through improving the structure and position of the teeth. Not all authorities are convinced that the above program is sufficient to accomplish the desired result of making the nation's teeth sound. An eminent dentist, speaking at a dinner

of dentists recently, made the assertion that most of the sound teeth in the world existed before the tooth brush was invented.

In England and in America we are in a stage of progress where repair and cleanliness are goals toward which school authorities and parents are being urged to work. This is essentially trying to put an attractive superstructure upon a faulty foundation. In its present form this movement for the preservation of teeth is founded upon a plan which will call for more and ever more investment of time, money and human effort without any prospect of relief from the burden. The senior author pointed out several years ago that the only prospect of relief from the present deplorable condition of the teeth in America was through giving attention to the problem of forming sound teeth during the period of growth. He stated that this involves the application of scientific nutrition to the regimen of the expectant and nursing mother and to the management of infants and children. This idea has been accepted by almost all dentists, and is now being emphasized as an outstanding feature of the campaign against tooth defects.

The importance of the diet is emphasized by the fact that the calcification of the first set of teeth is said to begin between the fourth and fifth months of pregnancy and the calcification of the first permanent molars shows a tremendous increase in the last third of prenatal life. It is clear that good teeth are determined, in great measure, before the child is born. It is true that serious damage may, and often does, occur to the teeth in postnatal life. The infectious diseases of childhood such as measles, scarlet fever, etc., may temporarily interrupt the development of the teeth, and when development is resumed a fault may be visible in the enamel to mark the event.

The researches of the last few years which have shown so clearly the possibility of influencing favorably or adversely the structure and quality of the bones through the character of the food supply have led investigators to question whether or not there may be an important dietetic factor operating in determining the poor quality of the teeth of modern Americans and Europeans. No other nations have so great an incidence of tooth defects and diseases, and of diseases involving the attaching tissues of the teeth. The widespread interest in the nutrition of children of all ages during the last decade has resulted in the accumulation of a large number of weight-height-age records. These, together with the clinical observation on skeletal defects,

defective musculature, anaemia, faulty posture, and other evidences of malnutrition, reveal the fact that a large percentage of our children are not properly developed, and in many cases for lack of a properly planned dietary. One would suspect that dietary defects which cause such marked inferiority of bones would likewise cause structural defects in the teeth which might make them susceptible to disease. Several efforts have been made to throw light on the many questions relating to dental problems by experimental work directed toward determining the effect of faulty diets in animals on the structure and health of the teeth.

Teeth of Primitive Peoples.—Weld (1795) in his book on "Manners and Customs in Philadelphia" stated that American-born men and women had decayed teeth at an early age. He thought this was due to the very general use of salted provisions. He eliminated climate as a causative agent, since the Negroes and Indians had sound teeth.

Catlin (1861) in his book "The Breath of Life" stated that it was a common custom among the Sioux tribes to collect the skulls after the scaffolds gave way on which the bodies had rested, and to preserve them in large circles on the ground. Catlin saw many of these and says: "I was forcibly struck with the almost incredibly small proportion of crania of children; and even more so, in the exceptional completeness and soundness (and total absence of malformation) of their beautiful sets of teeth, of all ages, which were scrupulously kept together, by the lower jaws being attached to the other bones of the head." "The teeth arrange themselves very regularly as the keys of a piano. Their teeth do not decay but preserve their soundness and enamel and powers of mastication to old age." He further stated: "The chief (of the party of 14 Ioway Indians who visited London with Catlin) informed me that one of the most striking peculiarities which all Indian tribes discovered amongst the white people was the derangement and absence of their teeth, and which they believed were destroyed by the number of lies passing over them."

Hrdlička (1908) reported that among Indians of the southwestern United States "Dental caries is not rare, though much less frequent than among the whites. It is usually a molar or premolar which is affected. Occasionally the gums or alveoli become involved, resulting in suppuration. Necrosis of importance was not seen in the living nor in the bones examined. Defects of the palate in an Indian of full blood have not been met with, and but one minor case of hare lip came to notice."

Hrdlička (personal communication) states that at the National Museum they have a large collection of Indian skulls and bones from the Eskimo of northwestern Greenland. These bones are remarkable for their good development and solidity; while the teeth of the Eskimo as a whole, including those of northwestern Greenland, are probably the best of any large group of people living to-day.

It is said that the Sheiks of the Sahara keep their teeth white by the use of the inner bark of the walnut tree. Their teeth are rubbed with this bark twice a day, and diseases of the teeth are said to be virtually unheard of in spite of the fact that they take a large amount of dates containing a high percentage of sugar.

Ottofy (1908, quoted by Howe) examined the teeth of Igorot children in the Philippine Islands and found that 68 per cent of them had perfect teeth, and the imperfections in the remaining 32 per cent were so slight that they would have escaped notice if casually inspected.

Wells (1919, quoted by Howe, 1920) reported that the Highlanders of Scotland are comparatively free from dental decay, but the people living in the Lowlands have much decay. Speaking of the excellent dental condition of the Highland Scotch he says: "This is largely accounted for by their simple diet of natural foods." On the other hand he says of the extremely poor dental condition of the Lowlanders: "The great reason for this, to my mind, is the diet of more refined foods." Howe remarks that every dentist is familiar with the fact that many of the Swedish girls who come to this country as domestics have excellent teeth when they arrive, but after eating our refined and cold storage foods their teeth rapidly decay.

Ruffer (1921) stated that abnormalities of dentition are not peculiar to modern times either in France, England or Egypt. Remains have been found in England (prehistoric) which show great abnormalities in the position of the teeth. He stated that caries in teeth from ancient remains have been discovered in so many places that it is legitimate to doubt whether there was ever an epoch in these countries when the human species was not cursed by toothache.

From the earliest time caries has occurred in human teeth in Nubia and in Upper and Lower Egypt. Ruffer quotes from an investigator who stated "Both in Nubia and Egypt the ordinary form of caries is exceedingly rare, in predynastic and proto-

dynastic people, and among the poorer classes it has never been common at all until modern times. . . . Dental caries became common as soon as the people learned luxury. In the time of the ancient Empire, excavated by the Hearst expedition at the Giza pyramids, more than 500 skeletons of the aristocrats of the time of the pyramid builders were brought to light, and in these bodies I found that tartar formation, dental caries and alveolar abscesses were at least as common as they are in modern Europe to-day, and at every subsequent period in Egyptian history one finds the same thing—the wide prevalence of dental diseases among the wealthy people of luxurious diet and the relative immunity from it among the poorer people who lived mainly on a coarse, uncooked diet."

Ruffer comments on the fact that in Egyptian cemeteries diseased teeth almost dropping out of abscessed cavities are very frequent. Carious teeth, which have caused extensive diseases, were apparently allowed to die without the relief which would have been afforded by a very simple operation. They found no teeth filled with gold or any other metal. The only set of artificial teeth was found in a Roman grave.

McClendon, Riley and Elwell (1923) reported that Dr. L. R. Sullivan of the American Museum of Natural History, and Dr. H. G. Chappel, examined prehistoric Hawaiian skulls and found nearly all of them belonging to individuals over 40 years of age to show gingival caries of the teeth. They found these in those persons buried before 1778, and hence before their contact with the whites. The principal food of the Hawaiians was poi, made from taro root. Since the calcium and phosphorus of this food is very low they attribute the faulty teeth to its excessive use.

Views on Etiology of Caries.—There have been many views expressed as to the etiology of caries of the teeth. Since it is not possible at the present time to speak with assurance concerning this matter, the more plausible of the views which have been presented will be mentioned here. Rumsey (1921) believed diet to be an important factor in the etiology of caries.

Seccombe (1921) stated that the essential factor in the formation of dental caries was the presence of carbohydrates in the diet. He found salivary stasis to be present with caries and was always accompanied by fermentation. A diet containing an excess of acid-forming elements he believed to predispose to caries. Caries without salivary calculus he attributed to overingestion of sugar. Indigestion, lack of mastication, or improper

diet he stated may give rise to a thickened saliva containing an excess of mucin. Over-ingestion, auto-intoxication or trauma he believes may result in gingivitis.

Mummery (1922) has given an extensive discussion of existing knowledge in regard to variations in the composition and structure of enamel, and the relationship of these to caries. He also gives an historical account of researches planned to produce artificial caries.

Teeth of American Children.—Rypins (1923) examined 1197 pre-school children of Kansas City, Missouri, between the ages of three and six years. He found that 27.2 per cent had dental caries, which averaged over one decayed tooth for each mouth examined, and almost four decayed teeth for each of the carious mouths. Of the teeth erupted 6.3 per cent were carious. These children were about ready to begin their schooling, so these findings represent definitely the condition they will present upon entering school. Once in school, absence from studies is a handicap to the pupil and an economic loss to the community. On the basis alone of trivialities associated with dental caries such as toothache, abscess, or time required for receiving dental attention, the problem is an important one. Considered in the light of consequences of major handicaps that can and do result from dental disease,—rheumatism (often called growing pains), heart disease, and mal-development—the problem is a distinctly serious one. Dental disease, therefore, plays a serious rôle in the lives of little school children and the economic consequences are obviously of importance.

Cross (1923) stated that "Investigation has shown that 96 per cent of the children coming to this country from the southern part of Europe have sound teeth, while it is well known that the teeth of 96 per cent of the American children are defective. Strange to say, too, the vast majority of the foreign children never knew what a tooth brush looked like until they entered American schools." He further stated that all adult dental diseases except pyorrhea are the result of poor tooth development or neglect of the proper diet and hygiene during childhood. Dead teeth, focal infections and diseases of the heart, kidneys and joints are the results of childhood neglect of the teeth. If caries could be eliminated it would prevent all these later complications.

Dr. P. S. Schenck (personal communication) gave us the following data relative to the condition of the teeth of white and colored children of Norfolk, Virginia. Their free dental clinics

FIG. 28.—Illustrates the extent to which the teeth have degenerated among Americans in recent times. The trouble lies in great measure in faulty nutrition during prenatal life, infancy and childhood. A satisfactory diet is essential for the development of a sound set of teeth. If the jaws and teeth are poorly developed they must throughout life be vulnerable to bacteria agencies.—Courtesy of The Forsyth Dental Clinic.

show that a very much smaller percentage of negro children have dental defects and also that a very much smaller percentage of negroes are treated in the clinics. He says that the negro child eats as soon as it can eat solid food quantities of greens, cabbage, turnip salad and vegetables. They do not have the sugars and fancy foods which the average white child eats. Their diet contains a larger percentage of lime, iron and other salts, than is the case with the white child.

Dr. J. A. Murphy (personal communication) reported the following data concerning the condition of the teeth of the white and colored pupils in the public schools of Washington, D. C., during the years 1916-1923. There were 47,744 white and 27,-968 colored pupils; 39,828 or 83.4 per cent of the white, and 24,705, or 88.3 per cent of the colored children were found to have carious teeth. The average number of cavities in temporary teeth of white pupils was 0.6 as compared with 1.0 for the colored. The average number of cavities in the permanent teeth was 1.3 for the white and 2.3 for the colored.

Dr. D. J. MacDonald (personal communication), who is familiar with the Island of Lewis, Scotland, informs us that the inhabitants of this island are for the most part crofter fishermen, *i.e.*, they cultivate a certain amount of land and also engage in fishing. Until about 50 years ago their diet consisted mainly of bread and porridge made from coarsely ground oatmeal and barley meal, potatoes, fish, eggs and milk. Since then, however, the consumption of white bread, tea and other articles of "cultivated diet" has greatly increased and although oatmeal is still very largely consumed it is no longer of the coarsely ground, home-milled variety, but is mostly imported. Very little meat is consumed even at the present time, except in the town area. The older people in the country districts have extraordinarily sound teeth and seldom require the services of a dentist. The same cannot be said of the younger generation although they compare favorably with young people in Glasgow.

Epstein (1923) has reported a very interesting paper calling attention to the increase in intermediate and secondary hemorrhages following the extraction of teeth in a large number of patients in Central Europe. He believes that this condition is due to the fact that they have been living on a faulty diet for several years.

Dental Disease in Olmstead County, Minnesota.—Mebane (1924) reported the results of an extensive examination of the

health condition of the school children of Olmstead County, Minnesota. About 3,000 children were examined. In 50 children with severe chest deformities (rachitic) the permanent teeth erupted within the limits of normal variation. Dental caries were the most common defect; under-weight and tonsillar disease were next. Dental caries were more common in city children, and children with dental caries were more often under-weight. Malocculsion was found in 59 per cent of the children examined; in 40 per cent the biting edges were irregular and often resembled Hutchinson's teeth; 5.1 per cent had rachitic deformities of the chest and 12 per cent had flat feet.

He further discusses the prevalence of caries of the teeth during childhood and adolescence. He believes that teeth decay more rapidly during and after febrile diseases, due to the fact that during illness the teeth are not likely to be kept clean and that elevation of temperature causes any residual food upon the teeth to decay more quickly and form a nucleus for beginning caries. He emphasizes the importance of the six year molars, in that they are the keystone of normal occlusion in the mouth and as such their preservation is essential. They often present deep incisura when first erupting which retain food easily and early decay along these crevises is common. For this reason he emphasizes cleanliness.

Preservation of Teeth.—For several years an extensive mouth hygiene program has been carried out under the direction of Dr. Fones in the schools of Bridgeport, Conn. He emphasizes the importance of taking whole cereal grains in the place of refined cereals, brown sugar in place of granulated sugar, and stresses the importance of mouth hygiene. He agrees that if teeth are given proper care and the children take a proper diet much can be done in improving the health conditions among them. He likewise stresses the importance of the diet of the pregnant mother and agrees that without satisfactory prenatal nutrition the teeth of the child will be faultily constructed.

Hellman (1923) expressed the view that acceleration of body growth has an adverse influence on the process of dentition. This is certainly not true of domestic or of laboratory animals. Furniss (1924) emphasized the necessity of cleanliness in teeth. He also discusses the relation of diet to teeth and believes that general nutrition is of importance. Lack of sufficient cleansing food and excess of soft glutinous substances which stick to the teeth and ferment with acid formation, he believes to be the cause of

enamel destruction. He believes that tooth decay is in large measure due to an undue consumption of sugars.

Bunting (1924) discussed the influence of prenatal feeding with special reference to bone and tooth development. He reported a very interesting case which is of special importance in this connection. A woman aged 25 years vomited continuously for 13 months, during which time she became pregnant and carried a child its full term. Although she was unable to retain solid food and became very emaciated, the child at birth weighed 7.5 lbs. He seemed perfectly developed and thrived under splendid postnatal care. When he was 4 years of age he had a perfect set of deciduous teeth well arranged, on fully developed arches, without any signs of dental caries. It is interesting to note that this mother, a primapara, prior to her pregnancy had no dental caries, but since that time she has developed several cavities. It would seem that the mother had suffered more with respect to teeth than did the child. Bunting states that although we cannot say that the child's teeth cannot be affected by nutritional faults of the mother during prenatal life, it is quite within the limits of possibility. Although prominent defects resulting from nutritional disturbances are not apparent in the deciduous teeth, stigmata may be produced on the permanent dental follicles which will influence the later tooth development. The fetal metabolism may be so altered that during postnatal infantile life the bones and teeth may be seriously affected, as shown by rickets and hypoplasia in the teeth.

Experimental Studies on Dental Problems.—M. Mellanby (1918) studied the formation and calcification of the teeth and jaws in dogs as affected by diet. She interpreted her results to mean that some substance or substances similar in distribution to vitamin A have a potent effect as regards calcification of the teeth. Later work (1923) showed that puppies fed during the period of development of the permanent teeth on diets deficient in vitamin A showed one or more of the following defects of the teeth and jaws: (1) thick and poorly calcified jaw bones and alveolar processes; (2) irregularity in the arrangement of the teeth, as a rule more noticeable in the lower incisors; (3) delay in and retardation of the eruption of the permanent teeth; (4) interference with the calcification of the enamel which is often either deficient in amount or defectively formed; (5) interference with the calcification of the dentine, which may be either small in amount or poorly calcified, containing varying numbers of inter-

globular spaces; (6) abnormal development of tissue at the gingival margins and the peridontal membrane.

The daily diet to which these pups were confined consisted of 175 c.c. of separated milk, white bread (70 per cent wheat) ad libitum, linseed oil 10 c.c., and yeast 10 gm. In addition one of the dogs received 5 gm. of meat per day, a second dog 10 gm. of meat per day, and a third dog 50 gm. of meat per day. From the seventh to the fifteenth week of the experimental period the dog receiving 10 gm. of meat was also given 10 gm. of butter fat daily. In commenting upon these dogs she stated that the enamel of most of the teeth was poor, especially in the case of the dog receiving 50 gm. of meat per day. This pup grew the fastest but had the worst teeth. The enamel was best in the teeth of the dog receiving the butter as well as 10 gm. of meat per day.

When the pups were given diets containing a sufficient amount of fat-soluble vitamins although in other respects the same as above, the jaws, alveolar processes and the teeth, were well calcified and the latter were regularly arranged. The following results with young pups she interprets as showing the relationship of the fat-soluble vitamins to other elements of the diet as far as the teeth are concerned: (1) diminishing the calcium in the food as well as the fat-soluble vitamins resulted in the formation of badly formed teeth; (2) diminishing the calcium in the food, when the fat-soluble vitamin content of the food was high, reduced the amount of calcification, especially that of the dentine of the teeth. The dentine which was laid down was badly formed; (3) increasing the calcium in the food, and diminishing the fat-soluble vitamins, resulted in teeth badly formed although better than under condition 1; (4) increasing the cereal in the diet, when the fat-soluble vitamins were deficient, resulted in worse formed teeth and jaws; (5) cereals differed in their detrimental action on tooth formation. Oatmeal was the worst of those examined, white flour and rice the best.

She stated that the addition of wheat germ to white flour to the extent of 20 per cent had a definitely bad effect on tooth formation when fat-soluble vitamins were deficient. When oatmeal was the cereal included in a diet deficient in fat-soluble vitamins the teeth produced were extraordinarily bad. She believes that as fat-soluble vitamins can overcome to a large extent a deficiency of calcium in the diet, so also can they neutralize the harmful effects of oatmeal. She (1923) further stated that there is a direct relationship between the structure of the teeth and caries,

those badly formed being more susceptible to disease, whereas those well formed were immune. She says that the structure of human teeth is probably to be controlled by dietetic factors in the same way that she believes she has shown to be true in dogs. She (1924) stated that when the diet is only "moderately defective" exposure to radiation has a decidedly beneficial effect on the calcification and structure of the teeth. She found that if the diet produced very defective teeth improvement by radiation was less, and if the diet was very unbalanced and defective then the radiations made the teeth even worse. When the radiations were of small value, owing to the pronounced dietetic defects, she stated that the latter could be completely antagonized by adding cod liver oil.

Mellanby, Pattison and Prowd (1924) reported experimental work with children in which they were fed diets comparable to those which Mellanby (1918) had shown in the case of puppies to result in the formation of faulty as well as of well-calcified teeth. The following table gives the daily diets which they fed to the children.

TABLE XXVIII

Diet	*A*	*B*	*C* *(Hospital Diet)*
Milk	2 to 1½ pints	¾ to ½ pint	1½ pints
Bread	2 to ½ oz.	5 oz.	Ad lib.
Fat	1 oz. (butter)	1 oz. (margarine except towards end)	1 oz. (margarine except towards end)
Sugar (including cooking)	1½ oz.	3 oz.	1½ oz. and jam, etc.
Oatmeal, groats or gruel		2 to 4 oz.	Occasionally
Rice, tapioca, etc.	½ oz.	½ oz.	½ oz.
Meat (cooked)	1½ oz.	1½ oz.	2½ oz.
Potatoes	2 oz.	4 oz.	4 oz.
Other vegetables	2 oz.	½ oz.	½ oz.
Cocoa	Made with milk	Made with water	Made with milk
Egg	1	Very little	Sometimes
Fruit, etc.	¼ orange, or ½ tomato, or swede juice 1 or 2 dr.	As in diet A	As in diet A
Cod liver oil	3 to 6 dr.		2 to 3 dr.

They interpreted the quality of the diets as follows: A. abundant, calcifying vitamin and calcium; a small amount of carbo-

hydrate, not including oatmeal; B. less calcifying vitamin and calcium; much carbohydrate, including oatmeal; C.—hospital diet—intermediate amount of vitamin A, calcium and carbohydrate, some oatmeal. It is difficult to understand why Mellanby and co-workers did not feed a diet containing an abundance of the calcifying vitamin (vitamin D) and calcium, and include oatmeal, so that their series of experiments would have been complete enough to warrant their deduction, viz., that oatmeal has a very injurious effect on teeth. Since their diet B contained a very small amount of milk, much oatmeal, and no cod liver oil, one would expect to have poor skeletal growth as well as faulty teeth.

Fermentation and Dental Caries.—Howe and Keniston (1918) reported studies on the salivary factor in relation to dental caries. They stated: "It would appear that the saliva of persons with teeth immune to caries varies, as does also the saliva of persons with carious teeth; that saliva may neutralize substances taken into the mouth, and that the average immune mouth has the greater power of neutralization; but the ratio of resting and activated saliva in immune mouths does not vary enough from that of carious mouths to prove that this ratio is indicative of the production and maintenance of immunity from caries in any individual."

Miller (quoted by Howe, 1920) believed that the fermentation of carbohydrate with the formation of lactic acid is the cause of tooth decay. He bases his theory upon the following experiment: Teeth were placed in a fermenting mixture of bread and saliva which was renewed from time to time so that it might not become alkaline. After 3 months there were defects in the teeth which could not be told macroscopically or microscopically from tooth decay. Bacteriologically his work was limited and he found no specific organism as the etiological factor for caries.

Howe (1920) repeated and extended this experiment. Teeth were placed in fermenting mixtures composed of maltose, lactose, saccharose, dextrin, white flour and bread. In some of the tubes saliva from individuals that had had extensive tooth decay were used, in others saliva from cases of no decay and in others saliva from mixed cases. After 6 months some of the teeth showed an etched condition, some a decalcified effect, and in others no change was discernible. The effects produced were in general similar to those produced on teeth which had been subjected to a weak

decalcifying agent. Howe remarks on the pronounced differences of various teeth.

Howe fed guinea pigs on diets containing large amounts of starches and sugars in order to see whether or not he could produce tooth decay in animals by fermentation. The animals were on the diets 6 months to a year. The sugars were eaten readily and adhered to the tooth surfaces constantly. The flora of the mouth became aciduric in character, but no effect could be detected in any of the teeth. For 3 or 4 months microorganisms were fed which had been isolated from caries. The growth was constantly present in the guinea pig's mouth, but no effect upon the teeth could be demonstrated. Howe has produced rather extensive effects in the teeth, in the alveolar process, and in the jaws, by feeding guinea pigs vitamin-deficient diets.

Howe (1921) demonstrated that when guinea pigs were fed a diet consisting almost wholly of fat-free milk and rolled oats decalcified areas are produced in the teeth, the alveolar processes, the bones of the skull, and also other bones of the body. It should be pointed out that this diet would produce scurvy in a guinea pig. Recalcification was produced when the animals were returned to a normal diet. In a later paper (1922) he analyzed the teeth of normal guinea pigs and of pigs fed a scorbutic diet. He found the scorbutic diet to produce extensive decalcification of the teeth and some parts of the bones. He stated that calcification occurred as the result of adding vitamin C to the diet. It may be well to emphasize that no one has as yet shown any relation between the vitamin C and calcification, but if the animals were given green vegetables as a source of vitamin C they were in reality also given vitamin D, which aids calcification. Grieves (1922) reported the results of feeding rats various types of diets. Although there did not appear to be any particular dietary deficiency associated definitely with the occurrence of dental caries he believes that a proper calcium to phosphorus relationship together with the vitamin D is most important for the formation of teeth, bones and attaching tissues.

Prinz (1921) reported the following results on the origin of salivary calculus. Teeth were imbedded in modeling compound and narrow spaces were left on the buccal and lingual sides beneath the gingival margins to simulate pyorrheal pockets. These were kept at a temperature of 40° and the pockets were filled daily with fresh saliva. At the end of 8 to 10 weeks the teeth were removed from the compound. They presented typical tartar

incrustations on their roots identical in every way with the salivary calculi occurring upon the teeth in the human mouth. The same results were obtained when this experiment was repeated using a synthetic saliva prepared from dried albumin, carbon dioxid and the following inorganic salts: KCl, K_2SO_4, K_3PO_4, Na_3PO_4, $Ca_3(PO_4)_2$ and $Mg_3(PO_4)_2$.

McIntosh, James and Barlow (1922) found a definite type of bacillus constantly present in carious material which they examined. These bacilli produced a high degree of acidity by the fermentation of carbohydrates. The average final pH value of 9 strains was 2.75 which is sufficient to decalcify teeth. Teeth left in contact with pure cultures of this bacillus over prolonged periods showed changes almost identical with those found in caries. The teeth showed erosion of the enamel with penetration of the dentinal tubules and the formation of liquefaction foci. They proposed to name the bacillus, B. acidophilus odontolyticus. They resemble in their resistance to and the formation of acid the acidophilus of Moro, but biologically there were several points of difference.

Hall (1923) made determinations of the pH changes in the saliva under various conditions. His experiments indicate that a carbohydrate residue favors fermentation with acid formation and a protein residue favors putrefaction with alkali formation. It seems probable that the microbial changes in the mouth depend primarily on the kind of pabulum as well as on the kind of organisms. He stated that halituous breath may or may not accompany salivary putrefaction.

Diet and Tooth Development in Dogs.—Marshall (1923) discussed the changes in the teeth of dogs resulting from deficient diets. He examined the teeth of the dogs used for experimental studies by Jones (1924). Jones fed dogs as follows: The basal diet consisted of bread, cooked lean beef, skimmed milk powder, butter fat and fresh orange juice made into a mush with distilled water. A salt mixture, the composition of which was based on ash analyses of cow's milk except that the phosphorus was omitted and different proportions of calcium were used, was added. The phosphorus content of the diets was varied by adding dibasic potassium phosphate to the above salt mixture and the degree of alkalinity regulated by the addition of HCl. There were several groups of puppies and different groups were fed different amounts of phosphorus.

Jones reported rickets in some of the dogs. Shipley (personal

communication), informs us that he has not seen in any of the many dog bones which have been sent to him for diagnosis pathological changes equivalent to human rickets. Many of the dogs from which the bones had been taken were believed by the investigators to be suffering from rickets. He finds the condition to be in general the phosphorus sclerosis described many years ago by Dibbelt, Lehnerdt, Stoeltzner and others. The diets which have been employed for the production of rickets in dogs have, almost without exception, not been properly constituted for the production of the disease in the rat. There have been as yet no preliminary studies of the nutritive requirements of the dog for these elements especially concerned with the development of rickets, so there exists no basis for interpreting the results which is comparable to what we have for the rat.

Man and dog are similar in that they both have deciduous and permanent teeth and the shedding of the deciduous teeth ordinarily occurs without any observable discomfort to the animal. Nine pups of the same litter were fed a restricted diet from September 25 until they were killed in May and June. They had had vitamins and sunlight, but apparently insufficient or an improperly constituted inorganic supply. An examination of the specimens revealed at least two abnormalities in the teeth: a marked delay in dentition and a relative absence of dentine although there was a normal amount of enamel. The deciduous teeth were apparently normal in size and shape but they were not shed in the usual manner. It was observed that in the permanent set the incisors, cuspids and molars of the mandible were more generally affected than were the corresponding teeth of the maxillary.

This was especially true in one of the animals. It was also noted that the lower permanent incisors were mal-posed and presented the appearance of arrested growth, so that the position in the arch, as well as the contour and size of the teeth was influenced. The peculiarity of the contour was shown by an area near the tip of the crown, which is bounded by a distinct constriction which served to differentiate the crown into two parts. It was obvious that the calcification of the tip of the tooth proceeded at first normally and then ceased altogether or at least became very retarded. This cessation of normal growth was characterized by a ring and a corresponding constriction which suggested the beginning of hyperplasia. Calcification was later resumed but the period of subnormal growth remained definitely

inscribed in the tooth structure. The right cuspid was anomalous, whereas the position of the latter was apparently normal, the changes in form already described were clearly manifest. When the maxilla was compared with the mandible it was found that these variations in the degree of calcification were less marked than in the upper jaw. As an outstanding feature, however, the retention of the deciduous teeth must be mentioned. Comparable defects were observed in the teeth of other dogs examined, which seem to point definitely to a profound effect of faulty diet on the development of the teeth. Marshall (1924) has discussed the several hypotheses which have been advanced to explain the etiology of dental caries.

Influence of Diet on Teeth and Bones.—Toverud (1923-24) made a somewhat detailed study of influence of diet on teeth and bones. He found the normal orthodentin to be largely substituted by osteodentin in the teeth of guinea pigs fed a scorbutic diet. The osteodentin incorporating degenerated odontoblasts he found to reach far down toward the apex of the tooth and there was a very narrow space of orthodentin surrounding it. The pulp tissue became so degenerated that one could scarcely see its normal elements. The process has its beginning with hemorrhages in the upper part of the pulp and extends down toward the apex. The dentin seemed to be more affected than the enamel.

He found a great reduction in the amount of total ash and also in the calcium oxid but an increase in the amount of magnesium in the teeth of scorbutic as compared with the teeth of normal animals.

He also studied the teeth of rats on faulty diets. The diet consisted of whole wheat 92, butter fat 5, a complete salt mixture but without calcium. The chemical analyses of the bones and teeth brought out marked differences between the animals fed a deficient diet as compared with the control animals. The total ash, calcium and phosphorus in the bones of treated adult rats were very much below those of normal adult rats. The magnesium content was distinctly increased. The chemical changes in the teeth were similar to those in the bones. He concludes that the resistance of the teeth against decay must lie in their structure and chemical composition. Changes in these two factors may be the first step in the process of decay. The changes may be quite small and be sufficient to render the tooth susceptible for a third factor which may be a local one, but which may be the final step in the process of decay.

Nutritional Factor in Preventive Dentistry.—From what has been said in this and other chapters it will be evident that certain peoples in the past, and certain people of the present time, have escaped the ravages of dental disease, which is now such a heavy burden in America and in certain European countries. The evidence presented points strongly to the failure to form teeth during the developmental period which have within themselves adequate barriers of defense against the agencies from without which would destroy them as the chief cause of tooth decay. Diseased teeth appear to result from the eating of foods which do not demand chewing and consequent exercise for the teeth; adherence of pasty foods to the surface of the tooth, with resulting fermentative decomposition with acid formation which etches the enamel; unhygienic condition of the mouth; probably also abnormal composition of the saliva which should protect the teeth, but under certain conditions may fail to do so.

The development of sound teeth, capable of resisting the destructive agencies mentioned, is essentially a dietary problem. The small jaws and crowding of the teeth so frequently seen in children of to-day is almost certainly the result of faulty skeletal development, and will be influenced by the dietary errors which favor the development of rickets. Mal-position favors food packs and the maintenance of foci of fermentation between the teeth.

CHAPTER XXIV

DIETARY HABITS OF MAN IN DIFFERENT PARTS OF THE WORLD

From what has been said in earlier chapters it will be readily understood that students of nutrition until the last few years must necessarily have had an inadequate understanding of the fundamental principles which underlie the science. Several writers were inspired with enthusiasm for special types of diets. Each had, therefore, a polyphemic eye for one or another nutrient principle as overshadowing others in importance.

The prevalence of skeletal defects such as rickets and diseases of the teeth are now extraordinarily common among certain peoples, and relatively rare among others. There is a widespread belief that these defects are on the increase, and that there is something in the dietary of modern, civilized man, especially in regions where commerce is most developed, which tends to favor physical deterioration. We have on the one hand the evidence gained through animal experimentation, in which diseased conditions seen in man are produced at will in the animal, to serve as an aid to interpreting the problem as to the importance of diet in preventing skeletal defects. On the other hand, we may study human experience as far as possible, with a view to discovering the kinds of food which peoples have subsisted upon at various times and in various parts of the world, and correlate with the findings the condition of their bones and teeth. We have brought together in this chapter certain information which has a bearing on this subject.

Primitive man ate everything he could secure which was edible, including the flesh of mammals, fish, birds and shellfish, also eggs and insects, as well as fruits, berries, fleshy roots, nuts, and a few other seeds of plants among which were the seeds of those grasses which have since been developed into our cereal crops. Huntington pointed out (1915) that extensive agriculture was impossible until after animals were domesticated. Such seed

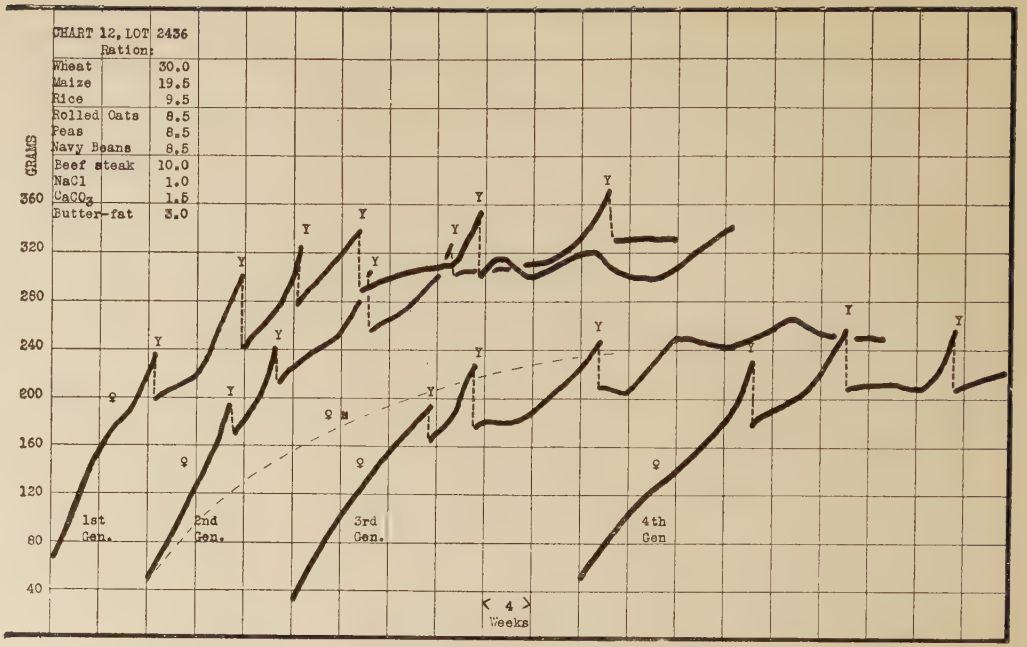


CHART XII.—This chart shows how good growth and high fertility may be secured in rats on a monotonous diet consisting of cereal grains, legume seeds and beefsteak supplemented with calcium, sodium and chlorine and with a source of vitamin A. If the inorganic additions are omitted the animals on this diet would be entirely unable to grow. If the butter fat were omitted they would be very inferior, and succeeding generations would be of low vitality. This would also tend markedly to reduce the fertility. If the beefsteak was omitted and no other supplementary source of protein was added in its place the animals would again be stunted, especially in the second generation. The omission of a supplementary source of protein would affect in a marked manner the capacity to produce and rear young. With the diet as tabulated, however, we have a complete food mixture and the family took it without any variation through successive generations without any striking deterioration in vitality.

as was produced was borne on isolated and scattered stems and was difficult to harvest in appreciable quantities. One exception was the wild rice which grew in the water and was protected to some extent from animals. Rice was harvested, therefore, from very early times, in parts of Asia and in some of the northern states of North America. Grass seeds of the type of the cereal grains were sought for at ripening by birds and the harvest time was always short. All the higher apes ate more or less of tender leaves which had mild flavors. After man reached the stage of development when food was regularly cooked he was able to eat coarse vegetables of the leafy type in greater variety and in larger amounts than when he had to eat them raw because of the difficulty of digesting some of them. Pot-herbs early became a regular part of the food of human beings as they advanced toward civilization.

Dietary Habits of Early Egyptians.—Ruffer (1921) states that the Egyptians were nicknamed "eaters of bread," and to them, as to the Hebrews, bread was synonymous with food. It was made from spelt, bearded wheat or barley. Cereals were cultivated from early times. Flour was prepared in the predynastic period by grinding grain between two stones or pounding it in a mortar. Ruffer comments that the teeth of the soldiers must have been severely tried by their daily ration of four pounds of bread because of its coarse texture. Attrition was intensified by the consumption of raw vegetables. Lentils, beans, artichokes, asparagus, beet root and cabbages were doubtless cooked before being served, but onions, cucumbers, garlic, radishes and turnips were eaten raw. The chief fruits of the Egyptians were grapes, figs, dates, dates from the dûm palm, pomegranates, melons, carobs, olives, apricots and the seeds of marshy plants. With the exception of dates and the seeds of the water plants the fruit eaten had little or no effect on the teeth, for although the rich people had large fruit gardens, Egypt has never been an extensive fruit growing country. Egyptians of the poorer class, like the fellah, probably tasted little fruit except dates and melons.

The diet was not wholly vegetarian for the predynastic refuse heaps of El Toukh have given many remnants of animals which served as food. Cattle were kept as far back as Egyptian civilization can be traced, and many wild animals and birds were kept in captivity and artificially fattened. The Nile and its canals were inexhaustible sources of fish. Salted and pickled fish were

favorite articles of food. The following inscription of Beha in the eighteenth dynasty refers to the diet of children. "The children were great and small-sixty . . . they all consumed 120 ephahas of durra, the milk of three cows, fifty-two goats, nine she asses, a hin of balsam, and two jars of oil." According to this the diet of the well-to-do children consisted in great measure of bread and durra, milk and oil. The poorer children were given simple cooked foods which could be grilled before the fire, roots and roots of plants growing in marshes, sometimes raw and sometimes roasted. The following is a quotation taken from an inscription during the nineteenth dynasty. "The king's messengers had good bread, ox flesh, wine, sweet oil, fat, honey, figs, fish and vegetables every day." The condition of the teeth of the ancient Egyptians living on these diets is described in Chapter XXIII.

Jews Originally Pastoral Nomads.—The Jews under the leadership of Abraham and of Lot migrated from the dry plains of Mesopotamia with their flocks and herds and appropriated to their own use the better watered lands of Palestine. They were a good example of a people who subsisted almost solely upon the products of an animal industry. They likewise illustrate an incident which has been many times repeated in human history, viz., a pastoral people who were sufficiently vigorous and aggressive to dispossess their neighbors of their lands. David, the self-reliant and brave youth, the great captain and the national hero, in whom all the noblest elements of the Hebrew genius were combined, was the product of a shepherd tribe.

Scant water supply makes the nomad's subsistence very precarious. It keeps him persistently on low rations, while the drought that burns his pastures and dries up his well brings him face to face with famine. According to Semple (1911) the daily food of the Bedouin is meal, cooked in camel's milk which has been soured, to which bread and meat are added only when guests arrive. The daily food of the shepherd agriculturist of the Kuen Lun margin of the Takla Makan desert is bread and milk; meat is used only three or four times a month. The Tartars eat all flesh of animals dying a natural death; in summer it is sundried for winter use because at that time the Tartars live almost exclusively upon mare's milk which is then abundant. Jenghis Kahn ruled that neither blood nor entrails nor any other part of the beast which might be eaten should be thrown away. Scarcity of food among the Tibetan and Mongolian nomads is reflected

in their habit of removing every particle of meat from the bone when eating. The privations and hardships of life in the desert and steppes discourage obesity. The Koko-nor Mongols of the Tibetan plateau are of a slight build, never fat. The Bedouin's physical ideal of a man is spare, sinewy, energetic and vigorous "lean-sided and thin" as an Arabian poet has expressed it. The Bedouins of Arabia buy flour, barley for horse feed, clothing and coffee, paying for them largely with butter and male colts. Harrison (1924) has written a most interesting account of his experiences in Arabia. He describes the Bedouin of the desert as "the lean, indefatigable nomad with the eyes of a falcon, but proud, hungry and indescribably dirty."

Diet of Pastoral Arab.—The diet of the pastoral Arabs consists in great measure of milk supplemented with moderate amounts of meat, cereals and dates. In settled agricultural districts pomegranates, melons and a few other things enter regularly into the food supply. The milk used is derived from goats, sheep and camels. It is soured promptly and eaten as curds, because of the impossibility of preserving it otherwise in a warm climate. Cheese and dried curds are also staple articles of diet. This regimen represents a diet largely of a carnivorous nature but supplemented with more or less vegetable foods. It is of the greatest importance to these people that their diet does contain much milk, since it is not possible for them, because of climatic conditions, to profitably grow farm crops. They could not subsist upon a meat, cereal and fruit diet without becoming inferior physically.

According to the Encyclopedia Britannica (vol. 2, p. 285, 11th Ed.) "Physically the Arabs are one of the strongest and noblest races of the world." Baron de Larrey, surgeon-general to Napoleon on his expedition to Egypt and Syria wrote: "Their physical structure is in all respects more perfect than that of Europeans; their organs of sense are exquisitely acute, their size above the average of men in general, their figure robust and elegant, their color brown; their intelligence proportionate to their physical perfection, and without doubt superior, other things being equal, to that of other nations." In the same article it is further stated of the Arabs that "Simple and abstemious in their habits they often reach an extreme yet healthy old age; nor is it common for them for the faculties of the mind to give way sooner than those of the body."

The use of very liberal amounts of milk is the rule among the people of Northern Africa, Arabia, Mesopotamia, the Balkan States and throughout wide areas in Asia. Wherever dairy animals are abundant in proportion to the population and their products form the staple articles of diet, fine physical development is seen without exception.

Douglas (1911) has written an interesting account of the use of fermented milks in the human dietary. The sour milks used so extensively throughout the Far East are variously designated as sheneena, leben, yohourt, kephir, koumiss, matzoon and dadhi. They are derived from the milk of goats, sheep, cows and mares. The fermentation is not purely a lactic acid formation through the transformation of the milk sugar, but includes an alcoholic fermentation as well. All these sour milks contain a relatively high acidity due to lactic acid, but more or less alcohol as well.

Tropical Agriculture.—Smith (1919) in discussing the world's food resources points out that the Panama Indians belong to another world, the tropic world. They have not developed from savagery through pastoral or animal-tending states. This is the Aryan method. The tropical people appear never to have used animals to any important extent, but developed a hand agriculture. They depended largely on starchy roots, sweet potato, yam, manioc, taro, caladium and other roots for food, which have been tilled so long that they have lost the power of producing seed. The banana, corn and sugar cane have been important additions to this untilled, soil-producing agriculture, which has, with the jungle, alternately occupied parts of tropical America for a length of time that no one knows. The tree crops have also offered great possibilities for food. In fact, tropic agriculture is to a surprising extent an agriculture of tree crops. Trees produce coffee, tea, most of the spices and cinchona. Trees give cocoa, coconut, palm nuts of several varieties and Brazil nuts. This latter is a source of fats which is becoming increasingly important. From the sago palm the natives make sago, a staple starch, and from the sugar palm in some parts of the tropics they also make sugar. The orange and avocado are also important fruits.

Indian Dietaries.—McCay (1912) reported an elaborate study of the diets of the peoples of various parts of India. He was led to the conclusion that a liberal supply of protein was the factor of greatest importance in determining the success of the human

dietary. It is now apparent that the protein moiety is but one of the several indispensable components of an adequate food supply, all of which are indispensable and must be viewed as of approximately equal importance. McCay's observations leave no room for doubt that the pastoral peoples of India are vastly superior in strength, health and manly qualities generally, to other classes of Indians. In those parts of India where the diet is largely derived from cereals, the physical development of the people appears to be the poorest. The rice eaters of Bengal illustrate this well. The details regarding the components of the diets of the different classes studied by McCay are not sufficiently complete to serve our present purpose, since he was interested primarily in the amount of protein which the different diets supplied. When his investigations were made it was not deemed of importance to list the individual food-stuffs eaten and their quantitative relations. This is regarded to-day as the most essential thing to know when one is called upon to evaluate a dietary. McCay's studies, however, based upon personal observations and on the reports of officers of the British Army to their Government concerning the fitness of the men in different parts of India for military service, form one of the strongest confirmations of the applicability of the principles of nutrition discussed in this book to human experience.

The most important milk product in India is ghee, or clarified butter obtained from the milk of buffaloes. It is sterilized for long periods so is more suitable than butter for the hot climate of India. It is usually so adulterated with fats and oils that Europeans prefer butter. India imports a fair amount of butter from Denmark, the United Kingdom and France. More condensed and preserved milk and cream than butter are imported, since many Indian women purchase condensed milks to feed their infants and children. In Bengal alone nearly two million people are supported by catching or selling fish. It is said that 90 per cent of the population of western India is dependent upon fish for sustenance.

The banana or plantain is cultivated throughout India except in the extreme northwest. The name banana is seldom used in India except by a few English people. All kinds of this fruit are indiscriminately called plantains. After mangoes, plantains are the commonest and most highly prized of Indian fruits.

Dietary Habits of Polar Eskimo.—One of the most interesting groups of people in the world from the dietary standpoint

are the Polar Eskimos. Their diet appears to be extremely poor in calcium and excessively rich in phosphorus, a relationship which works to the detriment of the skeletal system under ordinary dietary practices in temperate regions. They live on the northwest coast of Greenland, contiguous to the waters of Smith Sound and are the northernmost people in the world. Their food, fuel and clothing come almost solely from animals. Only rarely do they eat their native plants. Every animal supplies a part of the diet and no part of the carcass is thrown away. The land animals, although furnishing much of the clothing, constitute a relatively small part of the Eskimo food supply. The ptarmigan is the only land bird which plays any part in the dietary. It is upon the sea birds and sea animals that they depend for clothing and practically all their food and fuel (Ekblaw, 1921).

Ekblaw, who lived among the Polar Eskimo, states that fish are an insignificant part of the Eskimo diet, since they catch salmon only a short time in the year, and no other fish enters into their dietary. Caribou and musk oxen are not found about the Eskimo villages. Only at intervals of months or even years do the younger people of the tribe have an opportunity to eat the marrow of the bones of the land animals, since their chief food consists of animals from the sea, of whose bones they do not eat the marrow. They eat the liver of all animals except the polar bear, considering it a choice part of the animal. They are exceedingly fond of the thick, heavy skin of the narwahl and beluga and seem to need both of these in their food. The millions of dovekies and the thousands of murres, eiders and guillemots which frequent the coasts, supply an easy store of meat and eggs which can be readily put away for winter use when there is scarcity of game and consequent stress. The fact that the Eskimos of this polar tribe have such excellent physique, hair, teeth, such superb health, without any trace of scurvy, rickets or other evidences of malnutrition, is interesting in the light of their restricted and simple diet. Hrdlička (personal communication, 1924) has a large collection of skulls and bones from the Eskimo of northwestern Greenland. He states: "Their bones are remarkable for their good development and solidity; while the teeth of the Eskimo as a whole, including those of northwestern Greenland, are probably the best of any large group of people living to-day."

Diet of People of Faroe Islands.—Panum (1847) reported a most interesting account of the diet of the people of the Faroe

Islands. The only bread which the peasants ate was made of coarse barley which was made into long cakes or rolls without yeast. This bread and sour milk with mutton dried in the wind constituted the breakfasts. The mutton was dried in the wind and allowed to hang throughout the winter and by spring was regarded as fit to eat. Any unused portion of the carcass became filled with maggots in the summer, but this was regarded as improving the gamy flavor of the meat. This half rotten meat (flesh, fish or fowl) was called "rast." Large quantities of salted blubber of the Ca'ing whale were likewise eaten, as were cod's heads which formed a daily article of diet with them. The peeled stalk of Angelica archangelica were eaten as we would eat strawberries and cream. Panum saw men seventy years of age who had never lost a tooth, but in these cases the crowns of the teeth were worn quite flat as in many of the old British skulls.

Food of Lapps.—The Lapps are an excellent example of the success of a diet largely carnivorous. These people are scattered throughout northern Scandinavia and the Murman coast. They live almost entirely upon food derived from their reindeer herds and fish. They eat not only meat, fish and fowl, and eggs of wild birds in summer, but secure a fairly large amount of milk from their reindeer. They are a short and stocky people with good physical development and great physical endurance. They, like the Eskimo, must exert themselves greatly at times, in order to maintain themselves in their inhospitable land.

Nutrition of People of Iceland.—A most interesting experience is that of the people of Iceland. This island was settled in the ninth century by colonists from Ireland and Scandinavia. The colonists took with them cattle, sheep and horses. Their diet was practically carnivorous in nature for several hundred years. Martin Behaim (quoted by Burton, p. 32), writing of Iceland about 1500 A. D., stated: "In Iceland are found men eighty years old who have never tasted bread. In this country no corn grows, and in lieu of bread fish is eaten." Burton (1875, p. 154), quoting Pierce, states that rickets and caries of the teeth were almost unknown in Iceland in earlier times. Kerguelen (cited by Burton) has left an excellent description of the Iceland trade in 1767. The only money was fish and butter, while an ell of pig-tail (tobacco) equalled one fish. The fisheries were very extensive and would require four frigates to thoroughly protect them. Exports were included under salt meat, beef and

mutton, tallow, butter, wool, skins of sheep, foxes and seals, feathers, especially eider down, oil of whales, sharks and seals, fine and coarse jackets of Wadmal, woolen stockings and mitts, stock-fish and sulphur. The imports were fishing tackle, horse shoes, carpenter's woods, coffee, sugar, tobacco, snuff, beer, brandy, wine, dry goods, flour (wheat and rye), bread and biscuit.

As an index to the profound changes in the dietary habits of the Icelanders during the nineteenth century we may quote Burton, who visited the island in 1873, and who gave an account of the imports at that time. He states: "The cereals, whose consumption ranges from 24-30 bushels a head, are wheat and rye, in grain, flour, and biscuit." Again quoting Burton: "Besides cereals, the stores supply sugars, brown, candy, and white, hams, (rare and no potted meats), sausages and sardines, butter, figs, raisins, prunes and olive oil. The health conditions were good and dental caries were unknown until after about 1850. Stefànsson exhumed 96 skulls from a cemetery dating from the ninth to the thirteenth centuries and presented them to Harvard University. These have been described by Hooton (1918) who found no evidences of caries in any of them. There were but three or four defective teeth in the entire series, and these had suffered mechanical injury. During the last half century the incidence of caries has steadily increased in Iceland. This corresponds closely with the changed conditions with respect to importation of grains, and consequent changes in the character of the diet. It will be recalled that the grain eaters of ancient Egypt had very defective teeth (p. 478).

Diet of Rural Finland.—Sundström (1909) made a most valuable study in 1906-07 of the dietaries of the families of 103 farmers and tenant farmers in various parts of Finland. One family, representative of a class possessed of small land holdings, had nothing but bread, fish and sour milk during the seven days covered by the record. This family was typical of a group in which remarkable uniformity and monotony of the food supply prevailed.

A second type of household, representative of a class, had a diet somewhat better. The following menus were served:

Sunday.	B. Bacon with potatoes, bread, butter, milk. D. Meat, boiled rice, bread, butter, milk. S. Bacon, bread, butter, sour milk.
Monday.	B. Bacon, potatoes, bread, butter, milk. D. Bacon, bread, sour milk. S. Bacon, rye meal porridge, bread, milk.

Tuesday.	B. Bacon, porridge, bread, butter. D. Bacon, bread, butter, milk. S. Bacon, barley porridge, bread.
Wednesday.	B. Bacon, barley grits soup, bread, butter. D. Bacon, bread, sour milk. S. Oatmeal porridge, bread, butter, milk.
Thursday.	B. Bacon, potatoes, bread, butter. D. Pea soup, meat, bread, butter, milk. S. Pea soup, meat, bread, milk.
Friday.	B. Bacon, potatoes, bread, milk. D. Bacon, bread, sour milk. S. Bacon, grits-milk soup, bread, milk.
Saturday.	B. Bacon, potatoes, bread, milk. D. Bacon, potato soup, bread, milk. S. Rice porridge, bread, butter, milk.

A third dietary observed was that of a Swedish family in Finland. This shows a somewhat greater variety. During a seven day period this family had:

Bread—13 times	Potatoes—8 times	Curds—4 times
Milk—10 times	Grits-milk soup—4 times	Anchovies—4 times
Porridge—9 times	Meat—2 times	Blood omelet—once

No other food was served except milk sauce for the anchovies.

Another type of diet used by certain people in Finland is illustrated by a family who had potatoes every meal in the week (21 times), salt fish 9 times, fresh fish 4 times, porridge 4 times, bread 19 times, butter 8 times, milk 17 times, bacon 3 times, pancakes once, sausage once.

It is significant that in the Olympic games of 1924 the Finnish contestants carried away a disproportionately large number of honors, considering the population of their country.

Dietary Habits of People of the Hebrides.—A diet similar to the one used in Iceland has long been customary in certain islands in the Hebrides. The only agricultural crops which are successful are turnips and potatoes. Cattle are kept in sufficient numbers to give one or two cows to each family. Some oatmeal is available and forms a constant though not an abundant constituent of the diet. Many of the men are engaged in fishing and the unsalable parts of the cod form a regular and prominent part of the food supply. Milk, cod's head stuffed with cod's livers, turnips, potatoes and oatmeal constitute almost the sole food supply of the poor crofters. It has already been mentioned that rickets is rare among these people. McDonald (personal communication) states that the teeth of these islanders still adhering to the older dietary practices are far superior to those of Scotch people in the same climate but with the typical modern food supply.

Diet of Carnivorous Man and Animals.—Common observation shows that there are many examples of animals subsisting

entirely upon food derived from the tissues of other animals. The wolf, lion and tiger among mammals, and the eagle, owl and vulture among birds, are examples. Nowhere do we find more remarkable physical development than among this class of animals. It is obvious, therefore, that the tissues of one animal contain everything which is essential for the nutrition of another. Those who accepted the old ideas regarding the necessary components of an adequate diet saw no reason for distinguishing between different types of carnivorous diets. Dietary studies of the past few years have shown the muscle meats to have dietary properties very different from those of the glandular tissues. It has already been pointed out that muscle meats are very similar in their dietary properties to the cereal grains, and in order to make them complete foods it is necessary to supplement them with respect to calcium, sodium, chlorin, and three vitamins associated with the causation of the deficiency diseases.

The flesh eating of the carnivorous animals is very unlike that of civilized people in the temperate zone. The latter are very fond of the muscle cuts of meat, viz., steaks, chops and roasts, whereas carnivorous men and animals eat blood, glandular structures, bone marrow, fat and an appreciable amount of bone substance in preference to muscle tissue.

People's tastes vary greatly in the flavors of meats. At one extreme is the practice in the tropics of selling flesh within an hour or so after the animal is slaughtered. The meat is cooked when perfectly fresh. At the other extreme is the practice of hanging meat until it is putrid. Panum (1847) describes this in the Faroe Islands. Zenzinov (1924) describes the custom among the Tungas of northern Siberia of burying geese until they are putrid when they are eaten by people and fed to dogs. In the United States and in parts of Europe people prefer the flavor of meats which have been kept in cold storage for a considerable period. They undergo retrogressive changes and are extensively acted upon by bacteria which thrive at low temperatures. The rank flavor of gamy meats and that of fresh flesh are not liked by many people in the temperate zone.

The pronounced deficiencies of muscle tissue as a food-stuff suggested the question as to the reason for the success of the nutrition of strictly carnivorous animals. The explanation is found in the selection which these animals make of the parts of their prey. The custom of the carnivoræ is to open the jugular veins of a freshly killed animal and suck blood as long as it will

flow. This is what one sees when a rat or weasel raids a chicken coop. After blood they choose the glandular organs in temperate and tropical regions. In the Arctic region the second choice, or even the first, may be fat, depending on the previous state of nutrition of the animal. If it has recently ingested liberal amounts of a protein it may take only fat from the victim serving as its next repast. This is said to be a common practice among polar bears. A seal is often found by explorers, stripped of its fat but otherwise untouched. The demand for energy is so great in these bears that it is necessary to consume fat frequently and in large amounts. Since the protein metabolism is of much smaller magnitude, meat of either the glandular or the muscle type may be taken at much longer intervals.

Strictly Carnivorous Diets Adequate for Rats.—Blood, muscle or glandular organs taken alone will not support growth in a rat. A mixture of equal parts of blood and muscle fed to young rats enabled them to make a fair amount of growth but they remained stunted and failed early. (McCollum, Simmonds and Parsons, 1921.) Mixtures of blood, liver or kidney, muscle and bone, in the right proportions make a fairly satisfactory diet (McCollum and Simmonds, unpublished data.) The controlled experiment of the laboratory revealed the secret of success in the nutrition of a class of animals whose excellent physical development on a diet derived solely from animal tissues has often been interpreted as signifying that meat (muscle tissue) is a complete food, and one of exceptional value for promoting vigor. In reality the muscle cut of meat as the sole article of diet is the poorest part of the carcass. It is due to successful combinations which carnivorous man and animals make which renders their nutritional success possible.

Successful Rearing of Young Lions.—The importance of a proper choice of animal parts in order to make a complete diet is illustrated by the experience of those who have attempted to rear young lions in captivity. With few exceptions in the past such attempts have proven failures, since the animals developed faulty bones similar to rickets. If they survived they were very deformed and unfit for exhibition purposes. A young lion cannot grow normally when fed upon a piece of muscle meat with a large hard bone attached. When cubs are fed upon liver, fat and bone of a character such that they can eat a considerable amount, and when their diet is varied frequently with small animals which can be consumed entirely, such as rabbits or

pigeons, they grow to be strong and beautiful animals. In recent years young lions have been reared successfully by this procedure in several zoological gardens.

Scully describes the feeding habits of the lion in the jungle. He says that the zebra is the lion's favorite food. He opens the carcass at the flank, where the skin is thinnest, and eats the layers of skin and flesh covering the paunch and also the soft ends of the ribs, and the cartilages of the thorax. Then he removes the viscera without breaking the paunch. After eating the liver, kidneys, heart and lungs, together with any fat distributed among the viscera, he carries the paunch and entrails away and hides them. Then he returns and tears large pieces of meat from the thigh, which he swallows whole, skin and all. It is well known that the jackal feeds on the animal carcass which the lion has left. The jackal supplements this muscle tissue with eggs, and newly hatched chicks of the ostrich. This practice is so common that the ostrich and jackal are constantly associated in desert life. Since the eggs contain vitamins A and B, they supplement the muscle meats which the jackal obtains, and together with bone, complete his diet.

Toxic Products Produced from Protein.—Any strictly carnivorous diet must consist largely of protein and fat. When derived from the tissues of animals the carbohydrate moiety is so small and the protein content so high that the bacteria which flourish in the lower intestine will always be of the putrefactive type. The view of Metchnikoff that the body is injured by absorption of toxic substances formed in the intestine through the agency of putrefactive bacteria, rests upon evidence which can hardly be doubted. The beneficial effects resulting from taking a liberal milk diet, especially milk which has been soured by the B. acidophilus are attested by a great many people. A striking illustration of the manner in which a diet consisting largely of animal tissues modifies the bacterial flora of the intestine and influences the physical and psychical condition of the animals is given by Herter and Kendall (1909-10). They restricted monkeys to a diet of eggs, and cats to a diet of meat for one or two weeks, then changed them to a diet of milk and glucose. Previous studies had shown that eggs or meat encourage the growth of putrefactive organisms in the colon, whereas milk and glucose stimulate the growth of fermentative and lactic-acid-forming organisms. This is also true of a diet consisting largely of carbohydrates. After one or two weeks on

the milk and glucose diet the animals were returned to eggs and to meat respectively. These intervals were found sufficiently long to change entirely the character of the bacterial flora of the intestine.

As the proteolytic or putrefactive types of bacteria began to predominate, which occurred promptly after restricting the monkeys to eggs, the animals became sleepy and rested their heads upon their hands in a bowed position. They were stupid and responded slowly to external stimuli. They took their food very deliberately and manifested little interest in their surroundings. Not infrequently after a hearty meal they would spend much time trying to bite the woodwork of the cage. The urine was of small volume and amounted to approximately half that produced from the milk-carbohydrate diet. As the protein-digesting bacteria became established in the intestine, the amount of products of putrefaction in the urine increased markedly.

When the animals were changed to the milk-glucose diet both the psychical and physical attitudes underwent a great change. They no longer held their heads in their hands but assumed an erect posture. They were alert and bright, and showed a keen interest in their surroundings. Their appetites became sharp and the food was consumed with more avidity. The eyes, which were dull and lusterless while on the egg diet, became bright. They no longer attempted to chew the woodwork. The products of putrefaction almost disappeared from the urine. The evidence from human experience seems sufficient to warrant the conclusion that similar changes in the diet of man induce a similar modification in the flora of the intestinal tract. One may safely conclude that excessive consumption of meat with but small amounts of carbohydrates tends to promote the generation of toxic substances which induce lethargy in both man and animals.

Carnivora and Physical Activity.—It appears that the carnivorous peoples resemble the carnivorous animals in their attitude toward physical exertion. When well fed they are lethargic and remain idle, or do work that requires little exertion. They bestir themselves only when pressed by hunger. Dr. Grenfel states that the Eskimo is an inferior fisherman and will catch one fish to a white man's ten. This we may attribute in part at least to intoxication from poisons of bacterial origin due to putrefaction of protein in the intestine.

Dietary Habits of American Indians.—American Indians who inhabited the plains east of the Rocky Mountains and subsisted

mainly upon the bison were essentially carnivorous in their habits during the greater part of the year. Catlin (1832) visited a Mandan tribe of about 2,000 living in two permanent villages on the upper Missouri River. They had a fixed abode because they were too few to contend with the plains Indians, and accordingly practiced some agriculture. Nevertheless he states that "The buffalo meat, however, is the great staple and 'staff of life' in this country, and seldom, if ever, fails to afford them an abundant and wholesome means of subsistance. There are, from a fair computation, something like 250,000 Indians in these Western regions who live almost exclusively on the flesh of these animals, through every part of the year."

This tribe, because it did not wander, cultivated corn, pumpkins and squashes. Wild fruits and berries of different kinds were dried and laid away for the winter. An important article was the "pomme blanche," a kind of wild turnip which was much sought after by the migratory Indians. It is stated that the route taken in following the buffalo in their tribal hunts was often determined by the localities in which it was to be found in greatest abundance. It was eaten raw.

The numerically strong tribes migrated so as to keep in contact with the great buffalo herds, and so did not cultivate the soil. Accordingly they had vegetable food only as they gathered wild plants on the prairies, and fruits such as plums, choke cherries, wild grape, elderberries, wild raspberries, strawberries, etc., as well as the yellow lotus which grew in abundance in a few sheltered shallow bodies of water. Indian potato and falcata beans were also eaten. The latter produced two different fruits; pods containing small beans to which little attention was paid, and large subterranean beans which were eagerly sought as an article of food on account of their agreeable taste and nutritive value. These were collected by the meadow mice into hoards of a pint or more in a place, which the Sioux would seek out and take a part of, replacing the amount they took with corn.

The vegetable food of the hunting Indians formed an uncertain and variable proportion of their total food supply. They were essentially carnivorous. Reliable observers such as Lewis and Clark, Parkman and others testify to their being in many instances fine examples of physical development. Like the Eskimo they ate the entire edible part of the animals which served them as food.

Fletcher and La Flesche (27th Annual Report, Amer. Bur. of Ethnol.) described the method practiced by the Omaha Indians of stuffing the large intestine of the buffalo and roasting it before serving. The plains Indians lived almost exclusively on the flesh of the bison through every part of the year. During the summer and fall months they used the meat fresh and cooked in a variety of ways. During other parts of the year they ate principally pemican. This was made of buffalo meat cut in thin strips and dried very hard, and afterwards pounded in a large wooden mortar until it was made nearly as fine as sawdust, then packed in this dry state in bladders or sacks of skin. Marrow fat was collected from the buffalo bones and put into buffalo bladders for preservation. After it cooled it was very much like tallow but had nearly the flavor of the richest yellow butter. At feasts chunks of the marrow fat were cut off and eaten with the pemican.

Some of the Indians of the Southwest were more settled agriculturists. They engaged in hunting, but supplemented their animal food with more corn and beans, and ate more wild fruits and wild plants.

Reference has already been made to the perfection of the teeth of persons of advanced years among the plains Indians. Mr. V. W. McMullen informs us (personal communication) that in a series of about two hundred skulls excavated from the ruins of the old cliff dwellings, which represent the skulls of the ancestors of the Hopi, no carious teeth were found. It is evident that the development of the bones and teeth among these primitive people was far superior to those of the white people living in the same regions today. Hrdlička (1908), writing of the Indians of the Southwestern United States and Northern Mexico, stated "Dental caries is not rare, though much less frequent than among the whites. It is usually a molar or premolar that is affected." There is much reason to believe that changes in the diet since these Indians became wards of the Government have had much to do with the deterioration of their teeth.

Health of Non-Citizen Indian.—There is no better illustration of the soundness of the views regarding the types of diet which succeed in inducing good nutrition than the experience of the non-citizen Indian of the United States. All who observed the Indians in their primitive state agree that most of them were exceptional specimens of physical development. With few exceptions, however, during two generations they have de-

teriorated physically in a manner more marked than has the last generation of Americans of European stock. The reason for this is apparently brought to light by a consideration of the kind of food to which they have restricted themselves since they have lived on reservations.

There is no group of people with a higher incidence of tuberculosis than the non-citizen Indians. Wards of the Government, they have been provided with money and land but have in general shown little interest in agriculture. They have lived in idleness, and have derived their food supplies from the agency stores. In addition to muscle cuts of meat they have, therefore, taken large amounts of milled cereal products, syrup and molasses, sugar and canned foods, such as peas, corn, tomatoes. In other words, they have come to subsist essentially upon a milled cereal, sugar, tuber and meat diet. On such a regimen their teeth have rapidly become inferior, and are badly decayed. They suffer much from rheumatism and other troubles which result from focal infections. Faulty dietary habits are, in great measure, to be incriminated for their susceptibility to tuberculosis.

Other classes of Indians who have become successful farmers have not deteriorated as a result of contact with civilization except insofar as they have suffered from alcohol and venereal infections. The non-citizen Indian has suffered not because of contact with civilization, but because he has been forced into dietary habits which are faulty.

It is not essential that we retrogress to a state of social, educational or ethical inferiority in order to enjoy the physical fitness frequently seen in half savage peoples. Too much emphasis has been placed upon the beneficial effects from the standpoint of health, of hardship and exposure to which primitive man was subjected. Hardship and exposure never did any one any good. Protection from the elements is conducive to health as is also freedom from excessive exertion. One of the main factors which has tended to reduce civilized man to a state of physical inferiority as compared with his barbarous ancestors are changes in the character of his diet.

Successful Nutrition Among Leaf-eating Animals.—One of the greatest surprises in the history of nutrition research was the discovery by McCollum, Simmonds and Pitz of the unique dietary properties of the leaf as compared with those of the other parts of the plant. The knowledge gained by 1916 had shown

that the cereals, tubers, roots and legume seeds collectively, in any proportions, could not form a satisfactory food supply for any kind of animal with which experiments had been made. The thought naturally suggested itself that in the dietary of peoples who are largely vegetarian there must be something which supplemented the deficiencies of the seeds, tubers and roots. It was found that meats could not serve this purpose since they do not greatly enhance these storage products of the vegetable world with respect to several factors in which they are deficient. Later studies have clearly shown that the leafy vegetables play a very important rôle in the dietary of many Oriental peoples.

It is well known that grazing animals may live throughout their lives on grass or other palatable forage plants. The horse, cow, sheep, goat and deer, and other types of grazers, in their early history had no grain, but subsisted entirely upon grass and tender leaves. The bison never had anything throughout life but the buffalo grass.

The Chinese Diet.—Wang (1920) gives a very interesting discussion of the Chinese diet. It is one consisting of eggs, meat, fish, fruit, cereals and a great variety of vegetables. She states that rice takes the place of bread in China. Pork is used by practically all classes of people in all parts of China. Fresh pork is such a common food that wealthy people do not eat it. Lamb may be substituted for pork during festivals but beef is considered more or less sacred and is seldom used for food. The quantity of meat eaten is small but it is cut into small pieces and mixed with vegetables in a variety of ways. Fish and shell fish, including crabs, shrimps, lobsters and oysters, are used extensively.

Unlike Americans, who consider the muscle of an animal almost the only edible part, the Chinese people eat practically every bit of an animal except the hair. They eat the brain, spinal cord, the various organs, the blood, and even the skin. Blood is coagulated after it is drawn from the animal and can be purchased in the market in brownish cake-like pieces which look much like liver. Blood is an inexpensive food and is used liberally. Many chickens and ducks are also used. One can purchase in the markets such parts of the fowl as is desired, as duck's tongues, or a dozen duck's heads. Fowl are more expensive than are either pork or fish and are, therefore, seldom

used by the poor. They are consumed so extensively by the wealthy that they probably furnish the chief source of protein. Geese, pigeons, turkeys, pheasants and many other domestic and wild birds are also used, but owing to their scarcity they are considered as delicacies.

Eggs are used very freely in the Chinese diet. They use eggs of ducks and pigeons, as well as hens. They are fried and scrambled and in addition are used for seasoning, garnishing, making noodles and preserving. Eggs in one form or another enter into most Chinese dishes. A distinctly Chinese use of eggs is for preservation. At least three different kinds are produced, viz., hulidan, or salted duck's eggs, dsaudan or fermented eggs, and pidan, the so-called "Chinese old eggs."

It is partly this extensive use of eggs by the Chinese people which makes their diet adequate. They use little or no milk. Cows, as domestic animals, are employed only for draft purposes. Babies, invalids and the aged are sometimes given human milk. Mothers nurse their children for long periods, and then secure a wet nurse to prolong the nursing until the child is a year or two old. Vegetables are used much more frequently by the Chinese people than by Americans. In addition to spinach, cabbage, potatoes and radishes, many plants and weeds are eaten which are usually not considered food in America. Radish leaves, shepherd's purse, bamboo sprouts and a large number of sea weeds are used as foods. Since the Chinese people are lovers of vegetables they have developed great skill in cultivating them and in producing new kinds among them. There are at least six or seven varieties of spinach, differing from each other in the size of the plants, the size of the leaves, length of the stem and thickness of the leaf. One which grows most abundantly in central eastern China looks somewhat like cabbage.

There are no less than 20 varieties of soy bean, and from them thirty or more bean products are manufactured. Adolph (1922) has discussed in detail the many uses which the Chinese make of the soy bean. They regard it as a substitute for meat. He reported (1925) a study of North China dietaries. The data covers the diets of 304 adults and 114 children below 12 years of age, corresponding to a total of 19,777 adult unit days. A large proportion of the individuals studied were engaged in farming. The catty, in the following table is equivalent to 1.33 lbs.

TABLE XXIX

Foodstuff	*Weight in Catties*	*Foodstuff*	*Weight in Catties*
Wheat and wheat products	10,929	Corn	278
Melons (13 varieties)	7,270	Oils	246
Rice	6,207	Fish	188
Green vegetables (33 varieties)	2,207	Turnips, taro, etc.	156
Cabbage	963	Fruit (12 varieties)	155
Leeks	488	Shrimps and shell-fish	142
Onions	279	Sugar and starch	92
Garlic	116	Sweet potatoes	50
Celery	79	Beef, mutton, mule meat	44
Millet	1,877	Chestnuts, peanuts	36
Legumes (soy bean, etc.)	1,520	Milk (goats and cows)	33
Bean curd and other bean products	1,061	White potatoes	29
Kaoliang (Sorghum vulgare)	1,000	Chicken	28
Food accessories (salt, vinegar)	744	Other foods	15
Pork	319	Total	34,913
Eggs	287		

It will be recalled that Maxwell (page 367) observed many cases of osteomalacia in North China, the region in which normally the diet is represented by the above list of food-stuffs. The table represents the food of farmers. It is interesting to note that a class of people in this region who are "too poor to afford the necessities of life and too proud to work" had a high incidence of softening of the bones among their women, partly because their diet was restricted principally to cereal grains, and partly because they led sedentary lives and were little exposed to sunlight.

In the South and central parts of China where rice is produced abundantly it takes the place of wheat, but in the north in Pekin and Shantung wheat, corn and millet seed are used rather than rice. The rice is usually unpolished, since the polished is an expensive luxury. Ground or whole millet seed is made into cakes or a thin mush. It is eaten especially by children. In very poor families instead of meat and other ex-

pensive foods the ration consists chiefly of unpolished rice, or in the north millet seed or corn, bean curd, green onions, sometimes salt fish, and invariably a large quantity of leafy vegetables, including bean sprouts. The eggs, bean sprouts, blood and the liberal quantities of leafy vegetables, Dr. Wang concludes, have served as the "protective foods" in China.

Food of the Japanese.—Morimoto (1918) states that although rice is the important grain in Japan, the general belief that it is the principal food is true of the urban population only. The rural population use naked barley as their principal food. The soy bean is the most important legume in Japan. The Buddhists, forbidden to eat meat of any kind, early discovered that this legume would take the place of meat. The consumption per capita per annum is 0.386 bushels, compared with 5.32 bushels of rice. The daily consumption of cereals is, according to Morimoto, rice 0.117 gallons, barley 0.019 gallons, and other grains 0.010 gallons. The most important vegetables in Japan are sweet potatoes and a garden radish (daikon). Eighty and eight-tenths kilograms of sweet potato and 55.9 kgm. of radish are used per person per year. The radishes are commonly used as pickles, which are eaten with plain boiled rice.

Plums, mandarin oranges, persimmons and pears are the most important fruits in Japan. Animal protein is mainly derived from fish. Meat as a whole is not widely used enough to be called a national food-stuff. Almost two million people are engaged in fishing and more than 400 kinds of fish are caught in Japanese waters. The average amount of fish eaten per person per year is 17.625 kgm. or 49 gm. per day. Sea foods include both the animal and the plant group. The former includes the cuttle fish, the octopus and the shrimp. The latter includes two kinds of sea weeds. The consumption of beef is only about 1.535 lbs. per annum. The consumption of fresh milk per person per year does not amount to a quart. Morimoto states that 2,089,731 dozens of cans of condensed milk were used in one year. When one reckons the population of Japan as equivalent to 45,480,000 adults, this amount of condensed milk is insignificant. Other details concerning the Japanese diet will be found in the table on page 516.

Feeding Infants in China and Other Oriental Countries.—It will be apparent from what has been said of the Oriental diet

TABLE XXX

National Diet of Japanese People

	Consumption per man	
	Per year	*Per day*
Rice	5.32 bus.	.117 gals.
Barley (naked)	0.87 bus.	.019 gals.
Other grains	0.47 bus.	.010 gals.
Total (grains)	6.66 bus.	.146 gals.
Legumes	0.59 bus.	.102 gals.
Garden radishes	55.9 kg.	154 g.
Sweet potatoes	80.8 kg.	221 g.
White potatoes	15.4 kg.	41 g.
Other vegetables	56.7 kg.	155 g.
Total (vegetables)	208.8 kg.	571 g.
Fish	17.6 kg.	49 g.
Shell fish	2.2 kg.	6 g.
Seaweeds	1.2 kg.	3 g.
Other water products	1.8 kg.	5 g.
Total (water products)	22.8 kg.	63 g.
Beef and veal	1.58 lb.	1.9 g.
Pork, horse meat, mutton and goat meat	0.68 lb.	0.8 g.
Chicken	0.27 lb.	0.4 g.
Eggs (chicken and goose)	17.8 pieces	2.7 g.
Milk	1 qt.	....
Total (meat, fowl and milk)	.....	....
Fruits	13.5 kg.	37 g.
Sugar	14.1 lbs.	17 g.
Salt	39.6 lbs.	49 g.
Tea	0.7 kg.	1.9 g.
Alcoholic liquor	38 pints	0.44 gill
Tobacco	1.5 kg.	4.1 g.

Table taken from "The Standard of Living in Japan," by K. Morimoto, 1918.

that the amount of milk available for children is very small. Since the food is not suited for the proper nutrition of young children, it has been the practice of mothers to nurse their children for very long periods, 4 or 5 years being not unusual. The

early weaning of infants in Europe and America is possible because of the abundance of cow and goat milk. If the Orientals weaned their infants at 9 to 12 months of age, their infant mortality would be greater than it is. The diet of the Oriental child the first few years after weaning is in certain respects less satisfactory than that of children of the Western Hemisphere. The Chinese, Japanese and Filipinos have not had a supply of milk until recent years, when canned and powdered milks have been introduced.

Japanese children born in America have had a very different food supply from those in the mother country. Twenty years ago there was a great influx of "picture brides" into the Pacific coast states. We have, therefore, a large number of children in this country of Japanese parentage. Both boys and girls are larger at all ages than are Japanese children born and reared in Japan (Kanzaki, 1921). Without doubt it is the superior food which they have received which has made them outgrow their relatives across the ocean. The question has been raised on several occasions when the size of a people has been mentioned in connection with the nature of their food, whether size is not entirely a racial characteristic and inherited, rather than determined by such agencies as nutrition. We have some very good evidence on this point in the histories of our experimental rats which is described in a preceding chapter. When the nutrition of these animals fell just below a certain standard there was no easily observable sign of malnutrition. Their appearance and fertility remained such that they could have been judged by any one to be "normal," yet the size diminished from generation to generation. This is not set forth as an example of the inheritance of an acquired character. The inferiority of successive generations may be the result of injury during the nursing period, or due to confining them after weaning to the same inferior diet as that of the parents. The increase in size of Japanese children born in California and fed upon the products of its farms, over that of children of the same ages in Japan, harmonizes with the view. Horses introduced into the Shetland and Orkney Islands have degenerated in size, as have all other island horses. This is another illustration of the effects of a scanty and somewhat inadequate food supply upon animals. We are informed that Shetland ponies bred in America, and pastured on the rich fields of the middle western states, grow larger with each succeeding

generation. Roxas (1922) appreciates the principle under discussion as applying to the Filipino dietary. He suggests the importance of a greater and cheaper vegetable production, and also the fostering of the dairy industry as a means of improving the nutrition of the Filipinos.

Diet of People in Labrador. — Appleton (1921) has contributed valuable data on the diet of the people of Labrador. Under their dietary practices beri-beri, scurvy, edema and ophthalmia of dietary origin are of fairly common occurrence. She reported one case of pellagra. This is of extraordinary importance since it is very unlikely that the patient should have been exposed to pellagra, and its occurrence in this isolated region supports the view that faulty diet produces it. A most interesting point about Appleton's report is that sometimes one and sometimes another of the deficiency diseases develops among people whose diet is similar in different households. The entire population is evidently in a state of nutritional instability and small modifications in the diet determines which disease will appear. The following list of foods gives the approximate consumption of their principal articles of diet.

Bolted wheat flour	1¼ to 1½ barrels per person per year
Salt meats, pork or beef	1 or 2 barrels for a family of eight
Salt codfish	2 to 4 quintals " " " " "
Salt herring	1 to 3 barrels " " " " "
Molasses	160 gallons " " " " "
Potatoes	1 to 2 barrels " " " " "
Rutabagas	1 to 2 barrels " " " " "
Dried peas	20 to 40 gallons " " " " "
Raisins	10 to 20 pounds " " " " "
Butter substitute	160 pounds " " " " "
Condensed or evaporated milk	From a few tins to two cases
Tea	20 to 40 pounds per year for family of eight

Meat, salt fish, potatoes and dried peas are eaten only once or twice a week. Their supply is usually exhausted by April. Game is scarce. Fresh trout, cod and salmon are caught only during the summer. Gardening is little practiced, but in favorable seasons enough cabbage may be grown to last until November. A sack or so of onions may be added to the above list of foods. Poultry are not kept as a rule and egg powder is imported in sufficient amounts for making cakes for fêtes. The only fresh fruit eaten are partridge berries and a little yellow berry called locally "baked apple." Dock and Alexander plant which resemble spinach and parsley respectively could be used

as greens but they are little eaten. Cow's butter is used only by a few families. Tea is consumed in enormous amounts, 10 to 18 cups a day being not unusual.

It is interesting to note that spruce beer made from young spruce tips is a popular beverage in the spring. Early explorers in this region are said to have prevented scurvy by using a pine infusion. Another very surprising and interesting finding was the fact that popular belief attributes curative value to raw birds' liver for night blindness. Dr. Appleton does not appear to be convinced that night blindness has anything to do with vitamin A deficiency. She states "The treatment cures because the patient who submits to treatment remains in the house. Night blindness must be a retinal fatigue, rather than an inflammation as is the case with snow blindness, in which a photophobia is extreme. Poor diet may increase the possibility of seeing after-images just as fatigue does."

Diet of Italian People.—Mariotti (1919) has discussed the diet of the better class of Italians. Many kinds of cheeses are used in their cookery and extreme care is taken to develop special flavors in foods. For example, strawberries are never eaten with cream. They are not washed in water but in wine. They are served with claret or lemon juice and sugar. Peaches are soaked in white wine at the table. Sliced ham is served with fresh figs to bring out its flavor.

The peasantry live largely on cornmeal made into a mush or into a flat cake without yeast and baked between two hot stones. The Tuscan and Umbrian peasantry are said to live almost exclusively upon a vegetable soup. Beet root, cabbage, broccoli or potatoes are cut into small pieces and boiled in water to which is added a small slice of pork chopped very fine with an onion and some parsley. Tomato paste is added to this mixture. When served it has grated cheese sprinkled over it. It is said that only at Christmas and during the haying season do the peasantry drink wine or eat meat. No discussion of Italian food would be complete without mention of the macaroni. Macaroni is only one kind of what is called pasta. There are many varieties of this food-stuff, of which the more familiar examples are spaghetti and vermicelli.

Davis and Wood (1921) also reported on the food of the Italians. They state that wheat, corn and other cereals, vegetables, fruits, chickens, pigs and the milk of the goat constitute the food of the farmers. Fruits and vegetables are produced in

great quantities in Italy and even the poorest people have them in abundance. Children drink goat's milk, since every peasant has his goat or else his neighbor has such a supply that he is able to secure milk at a very low price. They consume very little meat, and that which is used is made into stews, with macaroni and tomato sauce. Eggs are used more abundantly than meat. Desserts of the Italian are frequently fruits. The Italians make their own cheese from goat's milk. They are very fond of eating green vegetables, and it is pointed out by social workers in the large American cities that Italians select in the markets a larger proportion of these than do other nationalities when they can afford them. The studies of Davis and Wood and of Gillett (1922) emphasize the fact that Italians in their own country have an abundance of goat's milk, cheese and green vegetables. In American cities they find milk to be expensive, as are also the green vegetables, and if economy is necessary they substitute coffee for milk. Since they have been accustomed to liberal amounts of Italian cheese they buy this cheese at a dollar and a half a pound rather than American cheeses at fifty cents a pound. A taste for sweets is soon acquired in this country, and they also come to use the refined cereal products since they are one of the cheapest foods. Gillett states "No milk and too much coffee and candy are the greatest factors of the nutritional problem of the malnourished Italian children." Mudge (1923) has also described her studies on the Italian dietary.

Phillips and Howell (1920) reported a very interesting paper on racial and other differences in dietary customs. They studied the Jewish, Italian and Negro dietaries. The following menus are typical of each.

Jewish Menus.—Breakfast: Coffee with milk and sugar, rolls and sweet butter. Lunch: Rye bread, with either smoked salmon or kippered herring. Supper: Soup with vegetables and meat, potatoes and rye bread.

Italian Menus.—Breakfast: Coffee with milk and sugar and bread. Lunch: Soup made with vegetables, with small pieces of onion and pork and Italian bread. Supper: codfish, cooked in oil, potatoes, Italian bread or spaghetti with tomato paste.

Negro Menus.—Breakfast: Pork chop, corn cakes, coffee and condensed milk. Lunch: Fried hominy, syrup and tea. Dinner: Fried ham, sweet potatoes, canned corn, bread and butter.

In commenting upon these studies they stated that the diet

of the Italian was characterized by a large amount of wheat products, green vegetables and olive oil; the diet of the negro by pork products and milled grains such as rice and hominy, whereas the Hebrew adhered more or less strictly to the dietary laws. An interesting feature of their studies was the calculation of the amounts of calcium, phosphorus and iron received.

DISTRIBUTION OF FAMILIES ACCORDING TO THE AMOUNT OF CALCIUM RECEIVED
(0.67 gm. per man per day taken as a standard)

Number of grams of calcium per day per man	*Number of families receiving specified amount of calcium*		
	Italian	*Jewish*	*Negro*
Less than 0.5 gram	7	8	12
From 0.50 gm. to 0.67 gm.	13	7	5
From 0.67 gm. to 0.84 gm.	10	5	5
From 0.84 gm. to 1.00 gm.	7	2	3
From 1.00 to 1.25 gm.	4	0	1
1.25 gm. or more	3	0	1

DISTRIBUTION OF FAMILIES ACCORDING TO THE AMOUNT OF PHOSPHORUS RECEIVED
(1.32 gm. per man per day taken as a standard)

Grams of phosphorus per man per day	*Number of families receiving specific number of grams of phosphorus*		
	Italian	*Jewish*	*Negro*
Less than 1.0 gram	6	3	8
From 1.0 to 1.32 gram	7	6	8
From 1.32 to 2.0 grams	23	9	9
2.0 grams or more	8	4	2

Sherman and Gillett found in a study of 92 families in New York City in 1917 that 48.9 per cent of the families were receiving less than the standard allowance of 1.44 gm. of phosphorus per man per day; 53.2 per cent were receiving less than 0.68

gm. of calcium and 41.3 per cent were receiving less than 15 mgm. of iron. This study in the winter of 1918 showed the following increased deficiency:

	1918 *Per Cent*	*1917* *Per Cent*
Families receiving less than the standard allowance of phosphorus	61.8	48.9
Families receiving less than the standard allowance of calcium	57.3	53.2
Families receiving less than the standard allowance of iron	51.2	41.3

Phillips and Howell in commenting on these studies say that doubtless this deficiency in mineral content was one of the causes for the undersize and lack of vigor noted among the children of the families studied. Among the Italian children there were many cases of rickets, in fact one would seldom see a child between 1 and 8 years of age who was not extremely bow legged. Sherman and Gillett found many homes in which they bought no milk because they thought it too expensive and at the same time were buying a small piece of cheese at a dollar and a quarter a pound.

The experimental studies on rickets and related conditions have brought to light the great importance of having a proper quantitative relationship between the calcium and phosphorus in the diet, since such a relationship greatly safeguards the developing skeleton. The most favorable relationship between these two elements demands a considerable excess of calcium over phosphorus in per cent of the diet. It is significant that Sherman and Gillett found in the human dietaries studied considerably more phosphorus than calcium. Such a dietary would be unfavorable to skeletal development in children. An abundance of vitamin D in the diet apparently affords safety to the child over a wide range of composition of the diet with respect to calcium and phosphorus.

Gillett (1922) has contrasted the dietaries of Italians, Jews, Lithuanians and Negroes. She cites meals in the table on pages 524 and 525, as well as discusses probable deficiencies and striking characteristics.

For many years Howe has observed the teeth of Italian immigrants arriving in Boston. He found the parents and children to have teeth far superior to those usually seen in Americans of the same age. Children born of these parents in America when they reached ages comparable to those of their older

brothers and sisters on arrival had teeth much poorer and more subject to decay. The teeth of the older children also deteriorated in America much more rapidly than those of children in Italy in the regions from which they came. Similar conditions prevail to a greater or lesser extent in the cases of immigrants from rural Ireland and Scandinavia. Many of the Italian children in America suffer from rickets, whereas those living under the conditions of their native country do not. Their housing conditions have changed. In their native land they lived out-of-doors during the greater part of the year. In the large cities they are living in the tenements with a consequent lack of fresh air, sunshine and with no place but the noisy, dirty street for the children to play.

The experimental facts presented in earlier chapters establish clearly that for animals the choice of food is of the greatest significance for growth, fertility and longevity. The special difficulty of securing a sufficient calcium supply, as well as sufficient of the several vitamins, emphasizes the element of safety which results from a proper selection of foods. It will be evident from what has been said of the dietaries of different primitive and less progressive peoples, that many of these have developed better, physically, than have many people of today who are living in the industrial regions of the world. Their teeth were better, with few exceptions, than those of modern Europeans and Americans. They escaped rickets in regions where now white children frequently have the disease. All people who lived long ago did not have good teeth, as is shown by the evidence of the Egyptian skulls of three thousand years ago. Their teeth in this particular region were of poor texture and did not last long. They suffered much from apical abscesses, and tooth decay was by no means uncommon. In seeking an explanation for this faulty dental structure we were led several years ago to suggest that the diet of the ancient Egyptians was not so good as that of many primitive peoples whose teeth were superior to theirs. An inquiry into the character of their food supply convinces one that this was true.

It has been pointed out on several occasions that the tendency now in the United States is to derive a relatively large part of the diet from refined cereal products, muscle meats, sugar and potatoes, a combination of foods which does not support satisfactory development, longevity and fertility in any animal with which experimental studies have been conducted. In discussing

TABLE XXXI

	Italian	*Jewish*	*Lithuanian*	*Negro*
Probable deficiencies	Calcium and vitamins	Iron and vitamins. In cases of financial pressure also calcium.	Iron and vitamins.	Calcium and vitamins.
One common dish	Beans, macaroni, tomato sauce and oil.	Gedemphts fleisch (pot roast made of brisket). Meat soaked according to dietary laws. Cooked with onions and seasoning in very small amount of water (in iron pot) for about 3 hours. Potatoes added one hour before time to serve.	Beef and pork, chopped fine, enclosed in a ball of dough 1 inch thick and boiled in salted water or soup 15 minutes.	Cush—leftover cornbread (soaked in water in which greens were cooked) fried with onions in pork fat.
Typical meals:				
Breakfast	White bread and coffee.	White bread or rolls; sweet butter, eggs or herring, potatoes, coffee. Milk for babies.	Rye bread and coffee.	Only two meals a day, and these whenever spirit moves.
Midday meal	Bread, fried peppers, eggs, ham or potatoes.	Smoked salmon, rye bread and butter, tea. (From the delicatessens.)	Soup, meat, potatoes, sauerkraut (or other sour food), rye bread, coffee or tea.	Fried pork, rice, soda biscuit, coffee.
Evening meal	Macaroni, beans, tomato sauce, oil.	Barley and mushroom soup, Gedemphts fleisch and potatoes, pickles or grated horse-radish, rye bread, tea. Compote of dried fruit, sometimes cake made without butter or milk.	Meat, bread, tomatoes (perhaps cake, canned fruit), tea.	Greens, salt meat, cornbread, sweet potatoes, buttermilk.

TABLE XXXI—*Continued*

	Italian	*Jewish*	*Lithuanian*	*Negro*
Striking characteristics (both desirable and undesirable)	Little meat. Large amount cheese and beans. Goat's milk in their own country—slow to use cow's milk here. Generous quantity of leafy vegetables, except when extreme economy is a factor. Few root vegetables or potatoes. Fruit if can be afforded. Oil—often a gallon a month. Much bread and macaroni—often 4-8 lbs. bread and 1 lb. macaroni daily. Strong prejudice against oatmeal and American cheese or oil. Much fried food. Taste for sweets acquired here.	Moderate amount of fresh meat and fresh-water fish. Large amount of smoked, salted, spiced beef, tongue or fish. Moderate amount of milk, cheese and eggs. Large quantities of legumes, potatoes, carrots and onions. Sorrel, beets, or beet greens in soup. Very few other green or fresh vegetables. Dried mushrooms Dried fruit, conserves and preserves used freely. Fresh fruit between meals. White or rye bread and rolls. Coarse cereals in soup. Sour salts in soup. Sour cream and sweet butter. Very little fried food. Food generally over-seasoned, over-rich, over-sharp or over-concentrated.	Chief foods: Smoked or salted meat or fish (often 4 lbs. a day). Sour milk, buttermilk. Sour salts in soup and pickles. Beets, tomatoes, onions (fried), potatoes. Few green vegetables or cereals. Considerable fruit. Moderate amount of fat and sugar. Rye bread.	Chief foods: Pork. Cornbread (often 3 times a day). Molasses. Cabbage and turnip greens. Lard or bacon drippings. Potatoes—sweet and white (always fried). Buttermilk in soda biscuits. Butter substitutes. Little milk. Melons.

Table taken from "Studies on Racial Dietary Habits," by Lucy Gillett—*Jour. of Home Econ.*, 1922, vol. xiv, 261.

present day dietary practices in America and their relation to defective nutrition, especially as shown by the prevalence of rickets and tooth decay, and malnutrition in children, it will be well to briefly discuss the changes in agriculture which have taken place in recent decades, since these are mainly responsible for the dietary changes which have been made.

Grass an Enemy to Agriculture.—Huntington pointed out that the great enemy of agriculture has always been grass. Primitive man could not compete with it, and accordingly could not practice agriculture extensively. The clam shell and the crooked stick were an ineffective armament for the early agriculturalist. When draft animals were domesticated he was capable of eradicating grass, but the great labor involved in harvesting and threshing cereals made their cultivation on a large scale difficult. Egypt was an especially suitable place for the growing of cereals with primitive implements, since the flooding of the land each year by the Nile prevented the formation of the tough sod which prevailed on grassy plains. It was for this reason that grains were grown in large quantities there from very early times.

Cereal grains may be kept in a dry state without deterioration for years. They served as a reserve food supply to be drawn upon during the less hospitable parts of the year and were a safeguard against famine. Joseph put away cereals in expectation of lean years. The amount of cereals eaten by many primitive peoples was relatively small, however. The Indians grew corn, but the amount often sufficed for little more than a great feast at the time it was ready for eating. The cereal grains at the present time furnish 35 to 45 per cent of the food supply in parts of Europe and America. This is an innovation in human experience. More and more cereals, meats of the muscle type, tubers and sugar have become the prominent articles of diet during recent years.

It was never possible to effectively deal with grass sod in farming until the invention of the modern steel faced plough. This invention was an American one dating about 1840. This type of plough cuts a ribbon of soil about a foot wide and three inches or more in depth, and turns it over with precision, exposing the roots of the grass to the drying action of the air. In this way it is comparatively easy to kill out the grass in the toughest sod. The plough of the type of the sharp stick which scratched the earth and tore it up in a rough, irregular manner

was an Aryan invention and is very old. The modern plough made possible the conversion within a few decades of the vast prairies of the United States and Canada, of Argentina, Australia, Southern Russia, Roumania, and other places into wheat or other grain fields. The world's production of cereals during the last seventy-five years has increased greatly with each succeeding decade, so that a greater proportion of the human diet can now be derived from this source than ever before.

The development of steam transportation in the second half of the nineteenth century was another factor of the greatest importance in stimulating cereal production and cereal consumption during the last 50 years. The railroad and steamship make it possible to transport grain from any part of the world where it can be produced economically to the great centers of population in the industrial areas. Without this it would never have been profitable to extend cereal cultivation as has been done. With the perfected agricultural implements such as the plough, the reaper, the binder and the thresher, which make for economy in grain growing, and with cheap long-distance transportation making a world market, the time necessary for the marketing of grains is so long that the problem of spoilage of flour became a very important one. For this reason inventive genius was turned toward solving the problem of milling a flour which would keep better than the whole ground kernel which had been produced through human history between the upper and nether millstones. This problem was effectively solved in 1879 when the modern roller mill process of milling wheat was perfected. In this process the kernel is not ground but is broken between rollers. The germ, bran and coarser grades of flour can be mechanically separated. The part of the kernel which has physical properties which make it crush between rollers so finely that it will pass through bolting cloth is marketed as the most desirable flour for human consumption. Such flour will keep in any climate without great commercial hazard, and hence is most desirable in the eyes of the manufacturer and distributor.

The appetizing qualities of meats are sufficient to insure for them a regular place in the diet of man wherever they can be afforded. The profit in sugar production has led to an enormous increase in sugar consumption in the United States and elsewhere. The consumption in this country per person per year is about nine times what it was a century ago. It is now about one hundred pounds per capita per year.

These facts are sufficient to convince one that the dietary practice of Americans and many Europeans have changed greatly within a few decades. We have been trying an experiment in human nutrition on a nation-wide scale, with a dietary which is of a kind which no people in history ever tried to live upon before. There was no way in which the results of such an experiment could have been foretold, for the dietary properties of individual foods were not understood, and the nutritive requirements of the body were essentially only half understood. It is possible, however, after approximately two generations of experience with a diet of the white bread, meat, sugar and potato type, with small, but generally inadequate additions of other foods of kinds which are capable of correcting the defects of the principal components of the food supply, to attribute certain unfortunate effects definitely to an unsatisfactory dietary. All the information available seems to warrant attributing in great measure the high incidence of malnutrition among children of pre-school and school ages, the faulty bone growth, bad teeth and faulty posture, to inadequacies in our national dietary, and to perverted appetite resulting from pampering and the formation of a liking for sweet foods.

CHAPTER XXV

THE RELATION OF THE DIET TO RESISTENCE TO DISEASE

Importance of Animal Experimentation to Human Welfare.—The sciences of medicine and surgery are built upon experience gained through animal experimentation. Most of our knowledge of the rôle which bacteria play in the production of disease has been gained through studies on the lower animals. Protective serums could never have been discovered except by such procedure.

The latest branch of medicine to develop is that of preventive medicine. This new field owes much to animal experimentation. Natural vitality enables the tissues to resist the entrance of certain bacteria. An optimal condition of the tissues constitutes an effective defense for the body against certain kinds of diseases. Our knowledge of the profound effect which the diet may exert on the vigor of the body is due to observations of the effects of specially planned experiments on small animals.

The study of the science of nutrition as a branch of preventive medicine has not been sufficiently appreciated in the past. It is true that the carbohydrate content of the diet of the diabetic has for many years been regulated as a therapeutic measure. Food has been selected with a view to the relief of constipation. Liquid or soft diets have been provided for the sick in certain diseases. Anti-scorbutic foods have been provided for persons who were subjected to conditions which experience had shown were likely to induce scurvy. The avoidance of excessive food consumption has been recommended. A low protein diet, a purin-free diet, sour milk as a therapeutic agent, and other dietetic regulations have been resorted to as means of affording nourishment specially suited to peculiar conditions.

Value of Scientific Nutrition in Preventive Medicine.—In recent years new and surprising relations between food and health have become apparent. Few would now reject the array of experimentally established facts and deny the relation of

beri-beri or scurvy to lack of some essential substances in the diet, or question that there is a high degree of specificity in the effects of the missing constituents which induce the development of these syndromes.

The researches in the field of nutrition have a greater value in preventive medicine in relation to raising the vitality of mankind, with all that this implies, than they have in the prevention of the occurrence of the deficiency diseases. This fact has never been sufficiently appreciated. In the aggregate, by far the most important effect of faulty nutrition in man is the result of errors of one kind or another which are not sufficiently grave to cause prompt and spectacular failure. It is the gradual operation of more or less constant, but unperceived causes, rather than of great exposures of an accidental nature which in most cases are responsible for undermining the health of the individual. Of these causes it now seems certain that the consumption of an improperly constituted diet is one of the most important. It is one of the causes of inferiority in physical development, instability of the nervous system, lack of recuperative power and endurance and consequent cumulative fatigue, and lack of resistance to infections such as tuberculosis and other types where specific immunity is not easily developed by the body. In addition to these the rate of development of senile characteristics and consequently the length of the span of life are greatly influenced by the type of diet to which one adheres.

Studies on Relation of Diet to Disease.—Immunologists and bacteriologists have recently become interested in the study of the relation of diets faulty in different ways to the ability of laboratory animals to react to the several kinds of stimuli with the production of those substances which are concerned with immunity to infections. A literature in this field is rapidly accumulating but the investigations yet recorded have in most cases the marks of immaturity, and are of a fragmentary rather than a comprehensive nature. It seemed best at this time to record briefly the experimental results available, but without any attempt to examine the results critically.

Smith (1913-14) found that epidemics of pneumonia due to the pneumococcus and B. bronchisepticus were very common in guinea pigs during the winter months when the diet was deficient in green food, while in the summer, when an abundance of green food was supplied, no cases of pneumonia developed. Hektoen (1914) reported that rats stunted because of a low protein con-

tent of the diet did not show any disturbance in the production of antibodies. Findlay and MacKenzie (1922) concluded from their studies that rats on diets deficient in vitamins A and B do not show any decrease in the opsonic activity of the serum. The phagocytic activity of the polymorphonuclear leucocytes of the guinea pigs with chronic scurvy was not increased. Hamburger and Goldschmidt (1922) examined the serum of children and of animals suffering from scurvy. They found this serum to contain the same amount of normal amboceptor and complement as the healthy controls. Hilgers (1922) found the complement titre in undernourished guinea pigs to be reduced. Koch and Smith (1924) reported that during scurvy a definite increase in the complement titre was observed over that of the fore period. During convalescence the titre remained somewhat higher than in the fore period. Starvation, they found, resulted in a lowering of the titre.

Zilva (1919) reported experiments designed to show the influence of poor nutrition on the production of agglutinins, complement and amboceptor. Guinea pigs were fed on an unrestricted mixed diet, a quantitatively restricted mixed diet, and a scorbutic diet. There appeared to be no difference in the amboceptor and agglutinating titres, nor in the complement activity of the blood. Rats were fed on diets low in calcium, low in iron, low in potassium, low in sodium, low in chlorine and low in phosphorus. They were fed diets low in protein, and also diets deficient in vitamins A and B. Although several of the deficiencies became manifest by restricted growth and poor condition of the animals, no reduction in the titres of the agglutinins or the amboceptor could be found in any of the groups except those receiving the low phosphorus diet.

Smith and Wason (1923) found a marked difference in the bactericidal titre of serum from animals on a rickets-producing diet as compared with that of normal animals. Complement titrations showed very little variations. Little correlation seemed possible between the complementing activity and the bactericidal value of the serum. They found the phagocytic indices to be lower in animals on a deficient diet. Werkman (1923) studied the influence of lack of vitamins on the production of specific agglutinins, precipitins, hemolysins and bacteriolysins in the rat, rabbit and pigeon. He found that rabbits and rats fed diets deficient in vitamins A or B showed no differences in their ability to produce agglutinins, precipitins, hemolysins or bacteriolysins.

Pigeons fed polished rice responded to the injection of antigen with the production of agglutinins. He also studied (1923) the influence of lack of vitamins on the resistance of the rat, rabbit and pigeon to bacterial infection. He found those animals suffering from pronounced vitamin deficiencies to be much less resistant to infection than were normal anmials. Rats and rabbits fed diets lacking vitamin A were less resistant to the anthrax bacillus and the pneumococcus. Rats suffering from lack of vitamin B likewise developed an increase in susceptibility. Pigeons fed diets low in vitamin B were also much more susceptible to the anthrax bacillus and the pneumococcus than were the control pigeons.

Relation of Vitamins A and B to Lymphoid Tissues.—Cramer, Drew and Mottram (1921) observed that absence of vitamin B from the diet of mice and of rats led to atrophy of the lymphoid tissue throughout the body and also to lymphopenia in the circulating blood. The number of polymorphonuclear leucocytes was not affected. The absence of vitamin A did not lead to atrophy of the lymphoid tissue and there was no lymphopenia. They found that absence of vitamin B led to characteristic nutritional disturbances such as loss of weight, emaciation, subnormal temperature, which may be designated by the term "marasmus." No such marasmic condition resulted from the lack of Vitamin A. This condition can be produced by other processes which destroy lymphocytes, such as X-ray exposure, indicating that the lymphocytes play an important rôle in nutrition. They conclude that vitamin B is necessary for the normal functioning of the lymphoid tissue.

Vitamin A and Blood Platelets.—Cramer, Drew and Mottram (1922) reported that a diet deficient in vitamin A produced in the rat a progressive decrease in the number of blood platelets. This thrombopenia was the only constant lesion for vitamin A deficiency and was characteristic of this deficiency just as a lymphopenia was characteristic of a deficiency of vitamin B. They found that the thrombopenia may be present in rats which are on a diet free from vitamin A before they show any obvious signs of ill health. When a profound thrombopenia had been established, addition of vitamin A to the diet produced a rapid increase of platelets to the normal number, provided the animal had not suffered too long and too severely from the vitamin deficiency. Exposure to radium produced a lymphopenia and also a thrombopenia if the dose was sufficiently large, but the animals

recovered rapidly if the radium treatment was discontinued and the dose had not been too large. They observed that when the number of platelets had been reduced below a certain critical level (approximately 300,000 in the rat) by exposure to radium or withholding vitamin A, the resistance of the animals to infection was greatly decreased, and infective conditions developed spontaneously and often gave rise to secondary anemia. These infective conditions cleared up when the number of platelets increased. These workers believe that the blood platelets have an important function in the mechanism of resistance to bacterial infection, and that alterations in local or general resistance to infection are associated with local or general changes in the distribution of the platelets.

Bedson and Zilva (1923) reported that rats fed diets deficient in vitamin A show a decrease in the number of platelets, but believed the decrease too small to be significant. Cramer, Drew and Mottram (1923) report that a diet free from vitamin B produces atrophy of the lymphoid tissue throughout the body and a lymphopenia just as radiations do. A diet free from vitamin A was found to produce a thrombopenia and when as the result of this finding, the behavior of blood platelets in radiated rats was studied it was found that the same lesion of thrombopenia could be produced if a sufficiently large dose of radium was applied. This thrombopenia was associated with the same general effects in both conditions, viz., an anaemia and a diminished resistance to infection. They also discuss atrophy of the intestinal epithelium. They criticize the work of Bedson and Zilva, asserting that the reason that they failed to duplicate the work of Cramer, Drew and Mottram, was that their technic was faulty. In their opinion Bedson and Zilva used an imperfect optical system which confused their results.

Bedson and Zilva (1923) respond with the assertion that they tried the optical system which Cramer and co-workers recommend and obtained the same results as with the system they had previously used. They conclude that they are unable to confirm the observations of Cramer and co-workers that a vitamin deficiency in the rat is productive of a severe thrombopenia.

Light and Formation of Platelet.—Cramer (1923) advanced the view that vitamins have a drug-like action on the alimentary tract. He believes these substances maintain the integrity of the apparatus for digestion and absorption. He believes that vitamin B has a specific stimulating influence or effect on the

lymphoid tissue; vitamin A on the intestinal mucous membrane as well as on the formation of platelets. Cramer and Drew (1923) kept rats in darkness on a vitamin A-poor diet and found a slight diminution in the number of blood platelets as compared with rats kept in a well-lighted room on a similar diet. The thrombopenia was sometimes associated with anaemia. Exposure to the mercury vapor lamp increased the number of platelets to normal and when anaemia was present also increased the number of red cells. They conclude that light may be a stimulus to the formation of blood platelets. They (1923) found light to be a stimulus to the formation of blood platelets but not an essential condition. Its absence could be counteracted by an abundance of vitamin A. The fact that conditions which stimulate the production of platelets will delay the development of certain bacterial infections supports the view that platelets represent a mechanism against bacterial invasion. Rennhard (1923) reported experiments with diffused light and with direct sunlight which clearly showed that development of yeast is more rapid in cultures exposed to the light. Since the same effect was obtained with the diffused light the possible influence of heat as a factor was excluded from these results. Yellow rays were somewhat more favorable than blue rays. The latter were rather injurious. Ultra-violet rays were somewhat inhibitory in their action.

Fat Absorption.—Mottram, Cramer and Drew (1922) made a histo-chemical study of fat absorption from the intestine. They demonstrated that the functional activity of the intestinal epithelium as regards absorption is profoundly affected by the presence in the food of vitamins A and B, particularly the latter. They believe that the vitamins have a stimulating action on intestinal absorption and probably also on intestinal digestion. In the absence of vitamins they found no delay in the passage of food in such animals. After exposure to radium sufficient to produce a lymphopenia the absorption of food was impaired in the same way as if vitamins A and B were absent, and this effect could not be counteracted by an abundant supply of vitamins in the food. These observations confirm the view previously expressed by the authors that lymphocytes play an important part in the absorption of food and the marasmus resulting from a deficiency of vitamin B is due to an impaired absorption and assimilation from the intestines.

Natural Vigor and Susceptibility to Tuberculosis.—There

are many who doubt whether the adherence to a faulty diet has anything to do with susceptibility to tuberculosis, but there is much reason to believe that it has. Experience has shown that there is no more effective therapeutic measure than proper feeding to raise the recuperative power of the patient. If such treatment affords greater prospect for throwing off the disease once it is established, it would be decidedly paradoxical to maintain that highly satisfactory nutrition could play no important rôle in protecting the individual against the establishment of the infection. Appleton has pointed out that the men of Labrador and Newfoundland suffer greatly from tuberculosis, although they spend at least half of the year out of doors in an invigorating climate. She states that owing to the strong winds which blow almost constantly during the winter, the houses are ventilated so thoroughly as to make garments wave which are hanging in the rooms for drying. She suggests that the faulty diet is the predisposing cause rather than unhygienic housing.

Effect of Diet on Experimental Tuberculosis.—Gloyne and Page (1921) observed that rats fed a diet deficient in vitamin A and innoculated with the tubercle bacillus died earlier than uninnoculated animals on the same diet. The deaths were due to non-tuberculous pneumonia and post-mortem examination revealed no naked eye lesions suggestive of tuberculosis. Tubercle bacilli were present at death in the spleen and blood of innoculated rats irrespective of the diets received.

Lange and Simmonds (1923) noted no significant difference in the reaction to infection of rats infected with bovine tubercle bacilli by subcutaneous innoculation on diets of varying protein content either in the general condition, weight curves, or in the gross or microscopic autopsy findings. Rats on a diet deficient in salts but otherwise adequate showed a more diffuse and extensive local reaction at the site of inoculation and a slower dissemination and a slower elimination of the lesions. Lange (1925) has continued the study of the effect of deficient diets on the susceptibility of the rat to experimental bovine tuberculosis. The course was essentially the same in the control and test diet groups with the single exception of the group on a diet high in fat but deficient in vitamins, which showed an earlier complete elimination of the tubercle bacilli.

Setti (1922) found pigeons which were fed polished rice to be less resistant to attenuated infections than were normal birds, and the latter developed more powerful immunity when vaccines were

employed. He interprets his experiments to show the relation that immunity and virulence of infections bear to the vitamin content of the diet. Stolte (1922) found that the ability of the organism to resist infection is reduced by a vitamin-deficient diet. He believes vitamin-rich foods to enhance resistance. Bickel (1922) stated that a vitamin-rich diet increases resistance and that diet is a factor in immunity.

Findlay (1923) observed that diets lacking in vitamin B produced in pigeons and rats definite changes in the hemopoietic tissues consisting of congestion and hemorrhage in the bone marrow, followed in the more chronic cases by a gelatinous degeneration. He noted that pigeons fed a diet lacking in vitamin B were much more susceptible to infection with the pneumococcus and the meningococcus-organisms to which they are naturally quite immune. They were also much more susceptible to infection with B. coli, and with B. enteritidis (Gaertner). The reduction of natural immunity he found to be related to the fall of body temperature produced by diets deficient in vitamin B. It was only marked when the cloacal temperature was lowered to 40° or below. He stated that the lowering of the body temperature appeared to decrease the resistance by (a) facilitating the growth of the invading organisms, (b) reducing the leucocytic response to the infection, (c) reducing the bacteriological power of the leucocytic exudate. He (1923) discussed the relation of vitamin C to infection. He reports experiments with guinea pigs infected with the pneumococcus, staphylococcus aureus, streptococcus haemolyticus, and B. coli. He demonstrated that animals suffering from a lack of the anti-scorbutic vitamin succumbed to a smaller infecting dose of bacteria than did the normal animals. Since in chronic scurvy there appears to be a degeneration in the hemapoietic bone marrow, this is suggested as being one of the factors responsible for the reduced resistance.

Mouriquand, Rochaix and Michel (1923) reported that guinea pigs on a scorbutic diet do not appear to assume the hemorrhagic character any more than do animals on a satisfactory diet when suffering from diphtherial infection. When infected with bacillus pyocyaneus and anthrax bacilli the development of lesions seemed to be more serious and more extensive in the control than in the under-nourished animals. Coulaud (1924) says the progress of experimental tuberculosis in guinea pigs is

surprisingly regular, and is greatly hastened by depriving the animals of vitamin C. Webster and Pritchett (1924) reported that the white mice from the Rockefeller Institute breeding room fed on the McCollum complete diet consisting of whole wheat 67.5, casein 15, milk powder 10, NaCl 1.0, $CaCO_3$ 1.5, and butter fat 5.0 per cent, are more resistant to mouse typhoid infection, mercury bichloride intoxication, and botulinus toxin, than are similar mice fed on bread and pasteurized milk supplemented by an oatmeal and buckwheat mixture, and dog biscuit.

Wertheimer and Wolfe (1921) studied the incidence and duration of infection, and mortality rates in several groups of children. Striking differences were observed which were all in favor of the adequately nourished children. These investigators believe that since antibody formation is a function of the cell any condition, such as marked undernourishment, which tends to impair the activity will be reflected in a decreased resistance of the body to infection.

Cramer (1922) stated that if children are fed too little of the vitamin-rich foods during infancy there is a lasting weakness which manifests itself if the child is exposed to a strain. He believes that there is a social aspect of the vitamin problem which has not been hitherto recognized, and that this is more important than the actual production of a severe vitamin deficiency. He suggests that the physical make-up of a community is determined largely by the ease and regularity with which an abundant supply of vitamins is secured for the pregnant and nursing mother and the nursing child. Many children are handicapped by physical defects, abnormality of form and lowered vitality as the result of failure to secure in infancy a diet adequate to meet their needs. These children represent borderline conditions of malnutrition. It is of great importance to appreciate the fact that there may occur by reason of faulty diet serious damage to the young without the development of symptoms which definitely mark the sufferer from a deficiency disease. The latter cases are the exception. Many malnourished children find their way into the school clinics. They are forced to repeat their work in one or more of the grades. They have teeth which are a source of lifelong annoyance. We do not desire to minimize the importance of after effects of infectious diseases as a factor in swelling the number in this group of children. In a very large number of cases, however, there can no longer be any

reasonable doubt that the most fundamental problem is that of securing for these children a wisely planned diet which will raise their vitality to a higher plane.

Impossible to State Minimum Requirement of One Factor Unless All Dietary Factors Evaluated.—Some of the confusion which is evident from the several studies which have been recorded in this chapter is probably to be accounted for through failure to control the experimental diets with sufficient care. We have reached the conclusion that it is not possible to state the amount of any food factor which constitutes the minimum or the optimum, or an excess, without taking into consideration the biological values of each of the other factors in the diet. The minimum amount of any dietary factor of constant quality which will just serve to maintain apparently normal well-being, will fail to suffice when another factor is modified so as to be unsatisfactory, and after such a modification of the diet increasing either of the two factors under consideration will suffice to protect the body from harm in some measure. There is a principle of relativity which is of fundamental importance in estimating the value of a diet as a whole. The diet must be considered as made up of a number of factors, and an effort made to adjust each factor at the optimum. Long observation of the type of experiments here described illustrates how animals may be brought into a "twilight zone" of nutritional instability and yet present no external evidence that they are not in the optimum condition.

The results of observing animals beyond the completion of growth to determine their fertility, success in rearing young and the rate at which they developed the characteristics of old age, were so striking that we early extended our observations to include restricting single families to a monotonous diet through several generations. This brought to light the fact that a diet may be sufficiently good to enable an animal to grow in a perfectly normal manner, and to exhibit a moderate amount of fertility, yet with each succeeding generation the progeny became smaller and less well developed physically, and after two, three or four generations the strain died out. Several comparable examples of similar experience in man may be cited. Campbell (1889) stated that among the poorer classes in the slums of English cities the family never passes beyond the third generation. Galton (1906) in a statistical analysis of one thousand town families and one thousand country families selected from

the town of Coventry and the surrounding rural population, found that the town population supplied to the succeeding generation but three-quarters of the number supplied by a similar number of country people. In two generations the adult grandchildren of the town dwellers amounted to but little more than half the number of descendants of the country dwellers. The employed urban dwellers are continually being recruited from country stock, and themselves tend to die out. Food is cheaper in rural districts and the selection and quality is distinctly better, generally speaking, than it is where the supply is derived almost entirely from the grocery store. The grocer carries a surprising proportion of stock derived from cereal grains, legume seeds, fruits, tubers and muscle meats. It has already been emphasized that food-stuffs from this list cannot be combined so as to constitute a satisfactory diet. The behavior of our rat population when restricted to diets corresponding in their origin to those of the urban dweller of either England or the United States is illuminating. The extent to which the rat has proven to be sensitive to even small modifications of the nature of its diet is truly amazing. It suggests that it is much more important than has been hitherto believed to have the diet nicely adjusted with respect to all its essential constituents, in order that the development shall be as nearly the optimum as possible. It is under such conditions that the characteristics of youth are preserved as long as possible and the span of life prolonged. We are taking too little of the protective foods, milk, the leafy vegetables, and fresh fruits and a movement toward stimulating the consumption of these classes of foods would go far toward effecting that improvement in our national health standards for which the many agencies concerned with the public health are seeking.

CHAPTER XXVI

NEW VIEWPOINTS IN HUMAN NUTRITION

In utilizing the results of nutrition studies on animals in interpreting human nutrition problems and their solution, the question of how far the results obtained with animals are applicable to the human being is a serious one. The period of growth in the human species is extraordinarily long as compared with that of the young of any mammals among the lower animals. This long period of youth doubtless is a factor of safety, since the opportunity for recovery from faulty nutrition at any time during early life is relatively great, because of the long time interval during which growth is possible.

We may reasonably set as our standard of optimal well-being in animals, uninterrupted growth to the full adult size, high fertility, low infant mortality, perfection of the bones and teeth, and the ability to resist infections and to reach advanced chronological age before senile characteristics appear. With such standards as a basis of judgment regarding quality of foods, we are justified in the generalization, on the basis of very numerous experimental results from many sources, that, for domestic animals, such as swine or rats, it is not possible to obtain optimal nutrition on any diet, the selection of which is restricted to cereal grains, such as wheat, corn, rice, etc.; the legume seeds, such as peas and beans; tubers and roots, such as the potato, sweet potato, radish, turnip, beet and carrot; and muscle meats, such as beefsteak. With any diet, restricted to these sources, in which highly refined cereal products, sugar, molasses, or other nearly pure carbohydrates, find a prominent place, the results to be expected in animal experiments will be less satisfactory than where these manufactured foods are replaced by their equivalent in whole cereals, and other vegetables of the types enumerated.

Experimental studies on animals have shown that milk and leaves of plants occupy a unique position among our available food-stuffs, in that they are so constituted as to correct, when suitable amounts are included in the diet, the defects of cereals, tubers, roots, and meats. For this reason it was suggested seven

years ago that they be distinguished by the term "protective foods."

It is unnecessary to dwell upon the specific nature of the deficiencies of each of our more important natural foods, but it may be said that in general there is a marked tendency for the diet of the average American of to-day to be deficient in the element calcium, to be somewhat over-rich in phosphorus in proportion to the amount of calcium it contains, to be deficient in the vitamin A, and in some cases in the vitamin C, as well as the antirachitic principle associated with certain fats, the vitamin D. Numerous experimental studies with animals have established the remarkable value as supplementary foods of either milk or leaves. This is one of the well-established facts in animal husbandry.

With such information available we sought six years ago an opportunity to test on human beings how far the principles established in animal experiments would apply to the nutrition of children.* With that end in view, we visited a considerable number of institutions where young children were being cared for, in order to discover if possible a group of children who, because of poverty or lack of knowledge on the part of those responsible for their care, were being fed a diet which we suspected to be inadequate for the promotion of growth or the maintenance of well-being. This search was rewarded by the discovery of an institution in which there were 236 negro children ranging in age from early infancy to 12 years, whose environment and food supply left little to be desired in relation to our objective. They were housed in a building of as good construction as one could desire. It was well ventilated, had good toilet facilities, sun porches where the children were instructed in bad weather, and about 10 acres of ground, partly woodland, where they could play out of doors in pleasant weather. Those in charge of the institution had the best possible attitude toward the welfare of the children, but the financial resources of the institution were meager. A casual inspection of the children in this orphanage showed that severe malnutrition existed in most of them. It appeared that if these children were actually suffering from malnutrition, the cause was probably due to the quality of their diet. Accordingly an opportunity was sought to improve the quality of the diet of these children in order to observe the extent

* The Institutional study reported here was made by the senior author in collaboration with Miss Helen T. Parsons and Miss Ethel Kalmbach.

to which they would respond in growth and well-being. This opportunity was granted, and a demonstration was undertaken with such funds as could be secured.

On ethical grounds it is not permissible to deliberately subject children to diets whose quality is suspected of being unsatisfactory, but it is not only permissible but commendable to improve the dietary of children who are, for reasons of poverty or lack of knowledge, being subjected to conditions inimical to their development. The demonstration which it was desired to make involved a considerable expenditure of funds. It was also desirable not to interfere to any considerable extent with the daily routine of the institution. These were both factors of great importance in determining upon the measures which should be taken to gain the information desired.

In 1919, 84 children between the ages of 4 and 10 years were selected for the study. The only basis of rejection was the obvious presence of syphilis. A considerable number of the children were believed to be tubercular. Many showed deformities characteristic of rickets. Since it was of the utmost importance to interfere with the daily routine of the institution as little as possible, and because of inadequate facilities in the kitchen, it was deemed inadvisable to attempt any additional cookery in connection with the demonstration. These 84 children were weighed, measured, and photographed without their clothing. Their ages were in practically all cases accurately known. Examination confirmed the conclusion that malnutrition of severe grade was widespread among the children.

A study of the dietary of the institution over a few weeks revealed the fact that it consisted essentially of a soup made of a fairly wide variety of food-stuffs, together with all the bread which the children cared to eat. Cereal was frequently served for breakfast. In Tables XXXII, XXXIII, and XXXIV, the food supply over intervals of one week is given in detail. This is fairly typical of the period covered by this study. All the components of the diet except the bread and some cereal and fruit were incorporated into the soup. The soup was palatable and attractive and was believed to possess a high food value. Actually from the standard of calories and proteins it left little to be desired. During the demonstration 4 to 5 per cent of the total calories of the diet was supplied in the form of meat, which was included in the soup, and went far toward adding to its attractiveness.

The diet of these children during the period when growth should be proceeding at a rapid rate consisted essentially of cereals, tubers, fleshy roots and muscle meats, and a considerable portion of the total food supply was furnished from a refined wheat flour in the form of white bread. Since the diet of bread and soup such as has been described contains no fresh raw food, the question at once arose why scurvy had not appeared among the children. They did not show any scorbutic tendencies, and the attending physician assured us that scurvy had not been a problem in the institution. The cause of this immunity to scurvy was soon discovered. Certain fruit vendors in near-by markets regularly donated lemons which were still sound but were likely to spoil before the next market day. It had been the custom to slice these lemons and give each child a slice. This one raw fruit was highly appreciated by the children and served as their source of the vitamin C.

The simplest procedure for improving the quality of this diet without involving any additional cookery appeared to be the addition of milk. This plan was adopted. Accordingly one group of 42 children received 1 quart of whole milk each per day during the demonstration period. This was prepared from Merrell-Soule's whole milk powder (Klim). It was prepared by dissolving the proper amounts of whole milk powder in cold water and agitating in a mechanical mixer. Only in very cold weather was the water warmed to some extent. The other group, which we designated as the check group, was continued on the institutional diet. We were thus in a position to compare the children on the two dietaries, since every effort was made in dividing the 84 children into two groups of 42 each to pair them so that any child in one group was quite comparable in age, size and condition to a child in the other group.

It is to be expected in any demonstration of this kind, where tubercular children, and children who had suffered from faulty skeletel development to a pronounced degree, were included, that certain individuals would be less capable of responding with growth to an improved dietary than would others. The accompanying charts give an accurate idea as to the general results of the comparison.

One of the striking features of the records of all children of both groups is the sharp increase in body weight, and at a more rapid rate than normal, in the first four to six weeks they were

(Continued on page 546.)

TABLE XXXII

(a) Milk Group

[Diet for two weeks; 40 children]

Food	*Weight*		*Calories*
	Lbs.	*Oz.*	
Hominy	4	12	7,638.0
Rice		10	944.0
Barley	21	0	33,810.0
Flour	1	12	2,805.4
Cornstarch	2	0	3,264.0
Corn meal	6	4	10,081.2
Green corn	1	1	487.7
Oatmeal	35	0	63,105.0
Bread	245	9	288,290.6
Dried beans	5	2	8,015.4
Salt mackerel	3	6	4,698.0
Beef	49	5	34,962.5
Ham	5	2	6,565.2
Pork		5	478.0
Potatoes	80	3	30,310.8
String beans	11	7	2,161.6
Carrots	5	1	1,037.8
Onions	4	6½	969.7
Turnips	1	15	347.0
Beets	4	7	927.7
Cabbage	3	13	544.7
Squash	2	1	443.0
Kohl-rabi	1	5	190.3
Preserves	12	0	19,020.0
Apples	5	0	1,070.0
Bananas	3	12	1,675.8
Condensed Milk		4⅔	448.0
Fat	2	0	6,820.0
Sugar		9⅓	1,029.4
Whole milk (dried)	1,190	0	.....
Total calories from institutional diet	.....		532,191.2
Calories from institutional diet per day per child	.....		950.30
Milk calories per child per day	.....		580.24
Calories per child per day	.....		1,530.54

TABLE XXXII—*Continued*

(b) INSTITUTIONAL DIET

[Diet for two weeks; 92 children]

Food	*Weight*		*Calories*
	Lbs.	*Oz.*	
Barley	8	12	14,087.2
Green corn	5	9	2,553.3
Oatmeal	70	0	126,210.0
Bread	1,165	0	1,367,710.0
Dried beans	2	13	4,398.1
Beef	56	11	40,191.3
Potatoes	1	11	637.6
String beans	19	9	3,697.2
Beets	8	0	1,672.0
Carrots	4	5	884.0
Onions	3	3	701.4
Turnips	1	3	212.6
Cabbage	4	9	652.1
Tomatoes	5	9	573.5
Eggplant	2	6	308.6
Squash	2	10	564.0
Kohl-rabi	2	8	362.48
Cheese	1	14	3,738.4
Condensed milk		9⅓	896.0
Preserves	24	0	38,040.0
Apples	34	8	7,383.2
Sugar		21	2,316.3
Total	.....		1,617,789.28
Calories per day per child	.....		1,256.04

TABLE XXXIII

(a) Milk Group

[Diet for two weeks; 40 children]

Food	*Weight*		*Calories*
	Lbs.	*Oz.*	
Hominy	6	8	10,452.5
Rice	9	5	14,816.0
Barley	17	7	28,074.2
Flour	4	0	6,412.0
Noodles	1	8	2,497.0
Corn meal	4	4	6,855.2
Corn	1	11	774.7
Oatmeal	34	4	61,752.4
Bread	242	2	284,254.8
Beef	69	14	49,541.2
Wieners	1	8	1,701.2
Potatoes	50	4	18,994.4
String beans	10	3	1,925.4
Green peas	1	3	538 9
Carrots	3	7	704.6
Onions	5	6	1,182.8
Turnips	2	8	447.6
Cabbage	3	15	562.5
Celery	2	15	246.0
Kohl-rabi	1	8	217.48
Eggplant	3	10	471.0
Preserves	12	0	19,020.0
Condensed milk		11⅓	1,088.0
Whole milk (dried)	140	0	.....
Fat	2	3	7,459.3
Sugar		9⅓	1,029.4
Herbs		3⅓	.....
Salt	.....		.....
Total calories from institutional diet	.....		521,014.58
Calories from institutional diet per day per child	.....		930.38
Milk calories per child per day	.....		580.24
Calories per child per day	.....		1,510.62

TABLE XXXIII—*Continued*

(b) INSTITUTIONAL DIET

[Diet for two weeks; 92 children]

Food	*Weight*		*Calories*
	Lbs.	*Oz.*	
Hominy		8	804.5
Rice	1	0	1,591.0
Barley	12	9	20,225.4
Flour	2	8	4,007.6
Oatmeal	69	8	125,307.8
Bread	1,312	0	1,540,288.0
Green corn		14	401.8
Dried beans	1	0	1,564.0
Fish	10	0	13,920.0
Beef	60	14	43,160.2
Potatoes	12	11	4,795.6
String beans	18	10	3,520.0
Carrots	2	14	389.2
Onions	3	14	853.2
Turnips	1	14	335.8
Cabbage	12	9	1,796.1
Eggplant	5	11	739.1
Squash		12	160.8
Preserves	24	0	38,040.0
Condensed milk		18⅔	1,792.0
Dripping		3	639.3
Sugar		14	1,544.2
Total	.....		1,806,075.6
Calories per day per child	.....		1,402.23

TABLE XXXIV

(a) Milk Group

[Diet for one week; 39 children]

Food	*Weight*		*Calories*
	Lbs.	*Oz.*	
Hominy	3	0	4,824.0
Rice	6	2	9,744.8
Barley	2	12	4,427.2
Flour		12	1,177.2
Macaroni		8	812.0
Oatmeal	11	0	19,833.0
Cornstarch	1	0	1,632.0
Corn meal	8	14	14,315.2
Bread	136	14	160,691.6
Dried peas		12	1,208.4
Liver		8	269.6
Pig tail		4	724.8
Veal	2	15	1,654.0
Beef	12	1	8,552.3
Potatoes	5	6	2,031.6
Carrots	3	1	627.8
Rutabaga		15	177.0
Turnips	5	14	1,051.8
Parsnips	4	2	1,237.4
Onions	2	0	440.0
Celery	1	10	136.0
Cabbage	6	4	893.6
Smoked cod	2	4	812.4
Sweet potatoes	3	12	2,092.8
Preserves	6	0	9,510.0
Bananas	4	14	2,178.6
Condensed milk		9⅓	896.0
Whole milk	595	0	.....
Fat	1	4	4,262.4
Sugar		5⅓	588.2
Salt		3	.....
Parsley	.....		.....
Total calories from institutional diet	.....		256,801.7
Calories from institutional diet per day per child	.....		940.6
Milk calories per child per day	.....		580.24
Calories per child per day	.....		1,520.84

TABLE XXXIV—*Continued*

(b) Institutional Diet

[Diet for one week; 93 children]

Food	*Weight*		*Calories*
	Lbs.	*Oz.*	
Hominy	10	0	16,080.0
Rice	2	0	3,182.0
Barley	5	0	8,050.0
Macaroni	1	2	1,827.0
Oatmeal	16	8	29,748.8
Corn meal	14	0	22,582.0
Dried peas	1	8	589,641.6
Bread	502	4	2,417.6
Lima beans	2	8	3,964.8
Kidney		9	183.6
Liver	4	10	2,489.0
Ear (as tripe)		10	163.0
Heart	3	13	5,032.5
Beef	36	0	22,524.0
Bologna		12	850.8
Bacon		12	1,947.6
Wieners	5	0	5,760.0
Corn beef	7	2	9,055.8
Potatoes	5	6	2,031.6
Dried beans	1	8	2,345.6
Dried lima beans	2	8	3,964.8
Carrots	4	0	820.0
Turnips	16	1	2,875.2
Salsify	1	11	.....
Parsnips	14	13	4,443.1
Onions	3	15	867.0
Cabbage	2	11	383.9
Celery		10	52.0
Eggplant		12	97.2
Salt mackerel	15	0	20,880.0
Sweet potatoes	17	14	9,974.6
Preserves	12	0	19,020.0
Bananas	11	10	5,196.0
Apples	6	8	1,391.2
Condensed milk	12	13	19,680.0
Fat		½	106.5
Sugar		7	793.8
Salt		0	.....
Parsley		3	.....
Total	.....		820,422.6
Calories per day per child from institutional diet	.....		1,260.2
Supplementary feedings:			
Sirup	.....		17,466.66
Sugar	.....		4,626.72
Bread	.....		38,021.2
Total	.....		60,114.58
Total calories per day per child	.....		1,492.3

under observation. This happened to both the milk-fed and check groups on the institutional diet. We can not determine with certainty the cause of this sharp increase in weight in both groups, but the most plausible explanation would seem to be the fact that our critical observation of the institution led to a certain increase in the total quantity of food provided to all the children of the institution just at this time. There is good reason to believe that before our advent the children were being fed not only a diet which was deficient in quality but likewise deficient in amount, so that their condition represented not only the results of malnutrition from a diet of faulty quality but the complication of partial inanition as well.

In the case of the check group, where the quality of the food remained essentially constant before and during the demonstration, the sharp increase in weight of 2 to 5 pounds was not continued. The weight curves of the children of this group suddenly flattened, and in practically all cases they remained nearly or quite stationary. In several children there was a gradual loss in weight during the first 12 to 15 months of the demonstration. There was only one child in the check group which made any appreciable gain during the first 15 months of the demonstration.

The milk-fed group showed marked contrast with the check group in several respects. In the first place, the sharp rise in body weight which was observed in both groups during the first few weeks was continued in most children throughout the demonstration, which for a considerable number of children covered a period of 21 months. One child of 5 years, who weighed but 28 pounds in the beginning, increased its body weight by 70 per cent at the end of 8 months on the milk, 90 per cent at the end of the first year, and reached a weight which was somewhat over the normal curve as given by the Holt, Boas and Burke table. Several of the children increased 50 per cent or more in weight during the first year.

There was likewise a marked change in the behavior of the milk-fed group as contrasted with the check group. The latter were apathetic and very tractable. The discipline of the institution was strict and these children were all quite obedient. Those in the milk-fed group, on the other hand, soon caused annoyance to their teachers by their restlessness and desire for activity. They also were frequently guilty of infractions of the rules.

The results of supplementing the institutional diet with milk were so striking that after 15 months it was decided to supple-

ment the diet of the check group with a quart of reconstituted milk per day, in order to demonstrate whether any of these children possessed the capacity to grow which they were not manifesting on the institutional diet. As was anticipated, during the next 6 months a considerable number of the check group grew at rates comparable to what had been observed in the original milk group. All things considered, the results of this demonstration constituted, we believe, a most satisfactory demonstration of the validity of the view that a dietary selected from cereals, tubers, fleshy roots and meat does not prove satisfactory for the physical development of young children. It shows further that milk is as effective a supplementary food for such a type of diet as has been repeatedly shown to be the case with experimental animals.

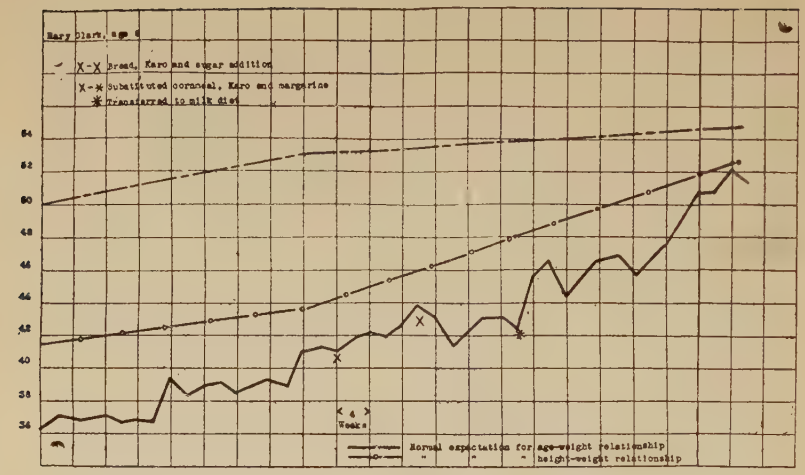
In making up these tables we selected three periods (two of two weeks each and one of one week) and gave the diet for the milk group and the diet of one of the check groups only. In the last set of tables we selected a period when supplementary feeding (sirup, sugar and bread) was being given to the check group.

A very few of the children were able to gain in weight on the institutional diet. Many who were not able to do so responded with growth at rates greater than the normal expectation, when the institutional diet was supplemented with a quart of milk per child per day. In a number of cases children grew well while receiving the milk addition, but declined in weight when after several months they were returned to the institutional diet. Several children who were unable to gain on the institutional diet grew at phenomenal rates with the milk supplement. There was no evidence in the records of any seasonal variation in the rate of growth of the children.

On the whole these data show that there was much difference in the vitality of different children. Some were able to grow on a diet which was obviously too poor to induce any growth in others who were less well endowed physically. Such a difference we have often seen in young animals. The results emphasize the fallacy of assuming that because a somewhat poorly selected dietary induces apparently normal development in one child, it is good enough for all children. The aim should be to plan the diet of the child so as to make it as nearly perfect as possible.

Observations on American School Children.—The children

(Continued on page 561.)



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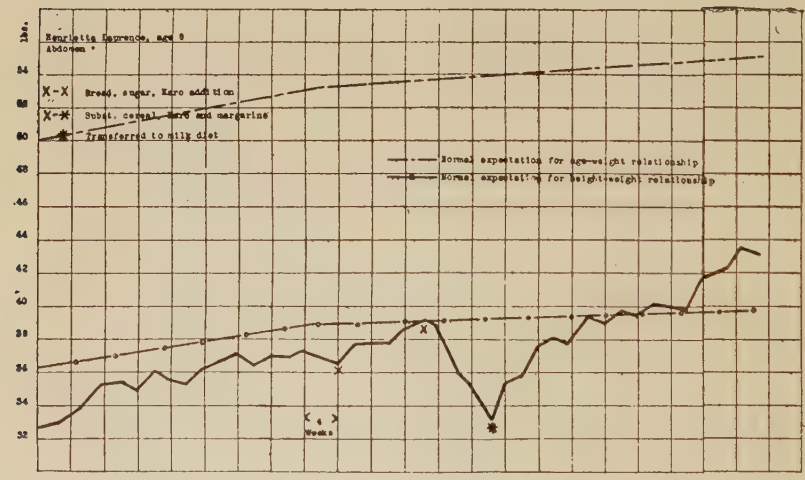
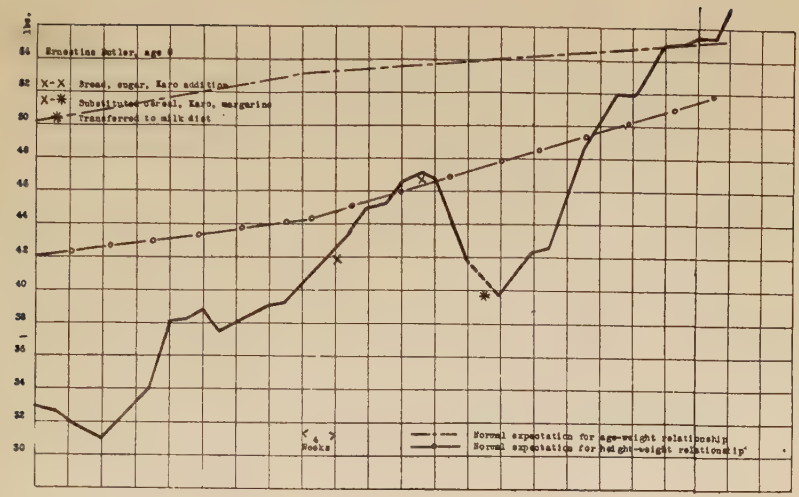
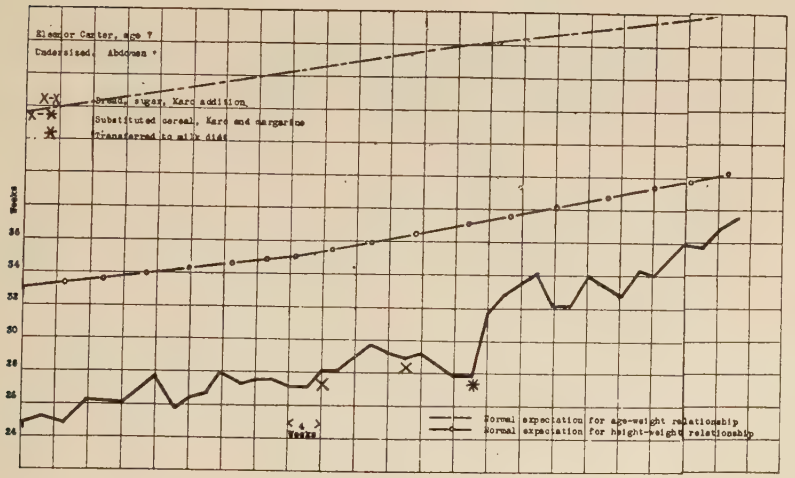


b

CHART 13—*a-b*. Original check group.—Received institutional diet plus supplementary feeding (for calories), but gained little or nothing until milk was added, when they grew. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve. * Transferred to milk diet.


Ernestine Butler, age 6
X-X Bread, sugar, Karo addition
X-* Substituted cereal, Karo, margarine
Transferred to milk diet
4 Weeks
Normal expectation for age-weight relationship
Normal expectation for height-weight relationship

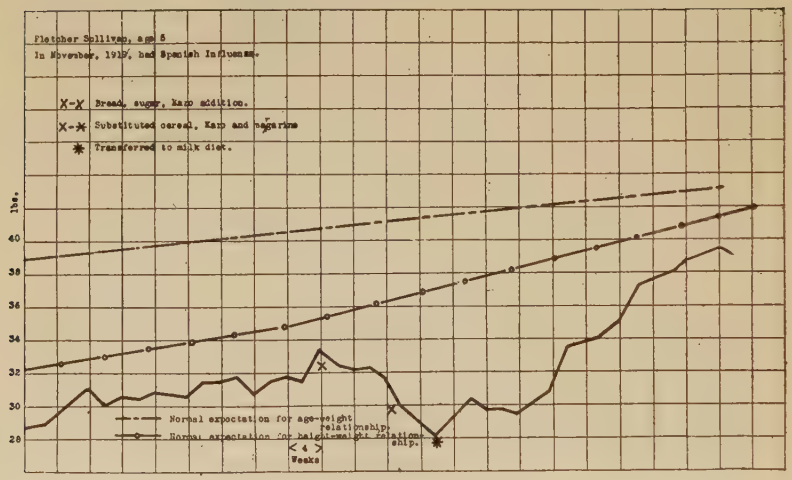
c



d

CHART 14—*c-d*. Original check group.—Received institutional diet plus supplementary feeding (for calories), but gained little or nothing until milk was added, when they grew. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve. * Transferred to milk diet.

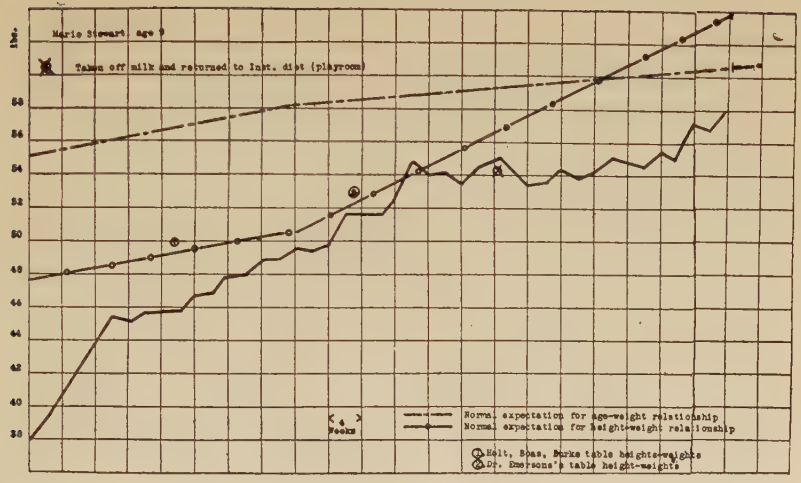
Original check group—Continued.



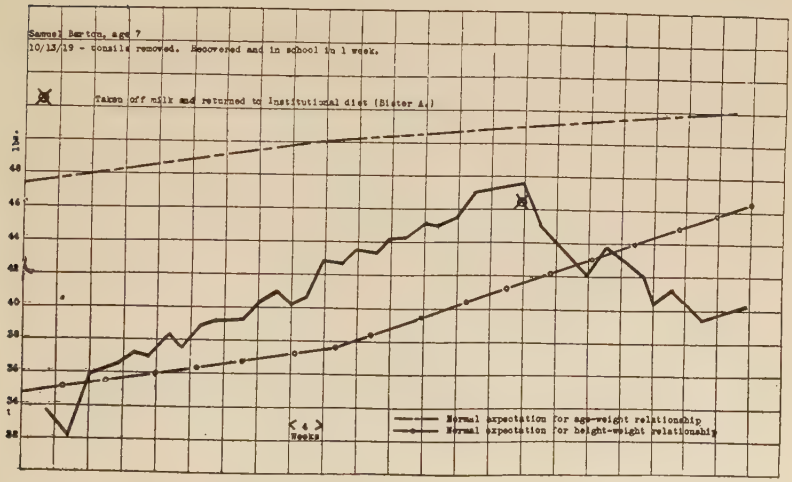
e

CHART 15—*e*. Original check group.—Received institutional diet plus supplementary feeding (for calories), but gained little or nothing until milk was added, when they grew. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve. * Transferred to milk diet.

Original milk group.

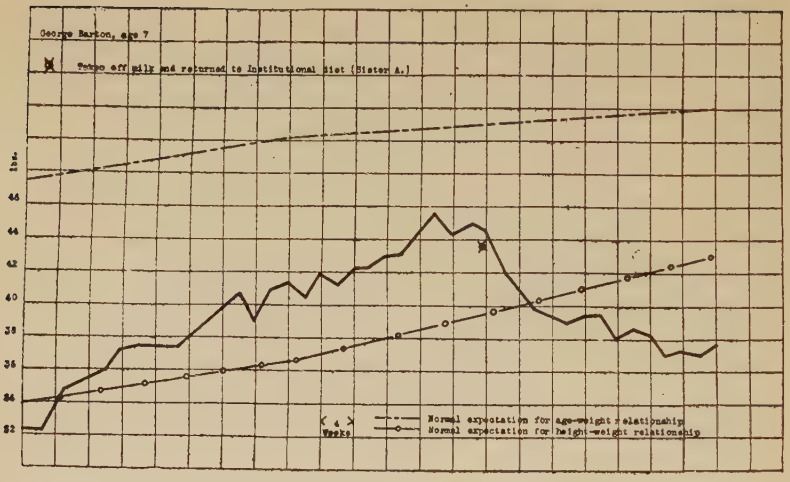


a

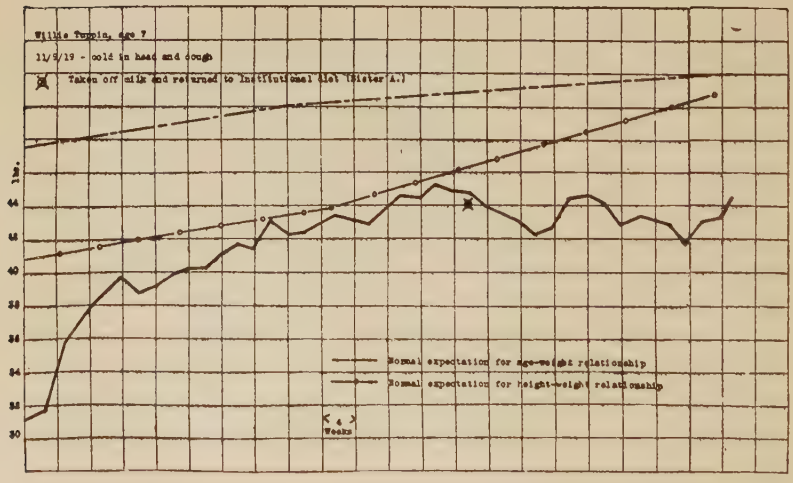


b

CHART 16.—*a-b*. Original milk group.—On milk diet for some months. When taken off milk diet and put on institutional diet, either lost weight, failed to gain, or gained but little. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve. * Taken off milk diet.



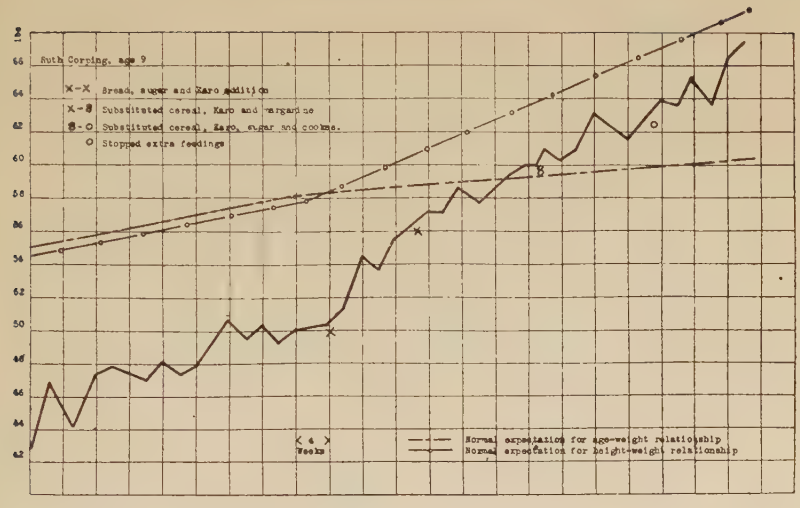
c



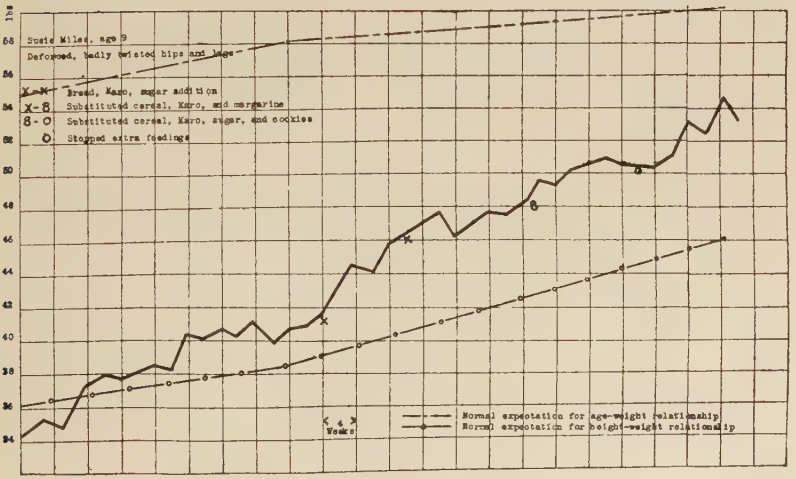
d

CHART 17—*c-d*. Original milk group.—On milk diet for 13½ months. When taken off milk diet and put on institutional diet, either lost weight, failed to gain, or gained but little. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve. * Taken off milk diet.

Original check group.


Ruth Corring, age 9
X–X Bread, sugar and Karo addition
X–8 Substituted cereal, Karo and margarine
8–O Substituted cereal, Karo, sugar and cookies.
O Stopped extra feedings
4 Weeks
Normal expectation for age-weight relationship
Normal expectation for height-weight relationship

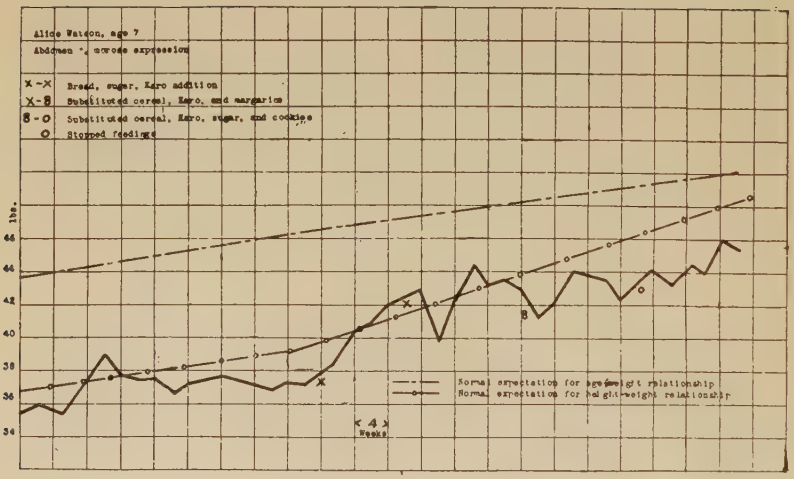
a


Susie Miles, age 9
Deformed, badly twisted hips and legs
X–X Bread, Karo, sugar addition
X–8 Substituted cereal, Karo, and margarine
8–O Substituted cereal, Karo, sugar, and cookies
O Stopped extra feedings
4 Weeks
Normal expectation for age-weight relationship
Normal expectation for height-weight relationship

b

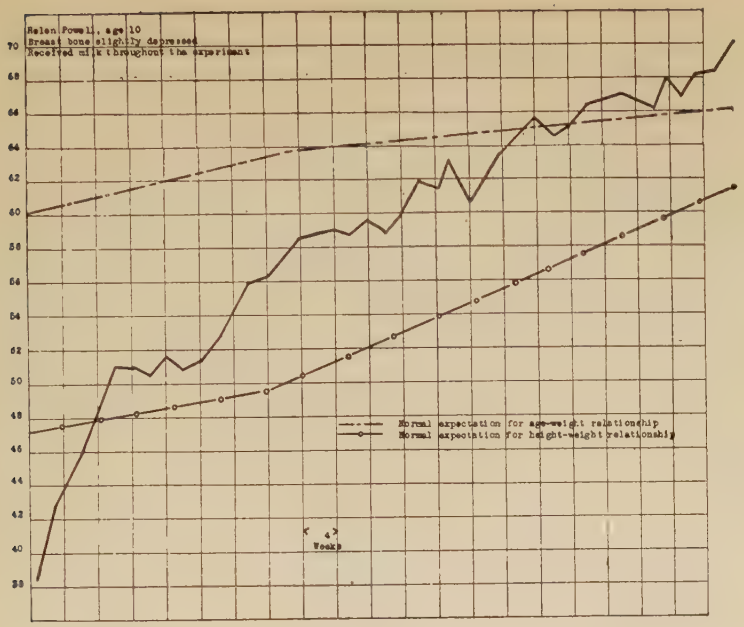
CHART 18—*a-b*. Original check group.—Received institutional diet plus supplementary feeding, and gained. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve.

Original check group—Continued.

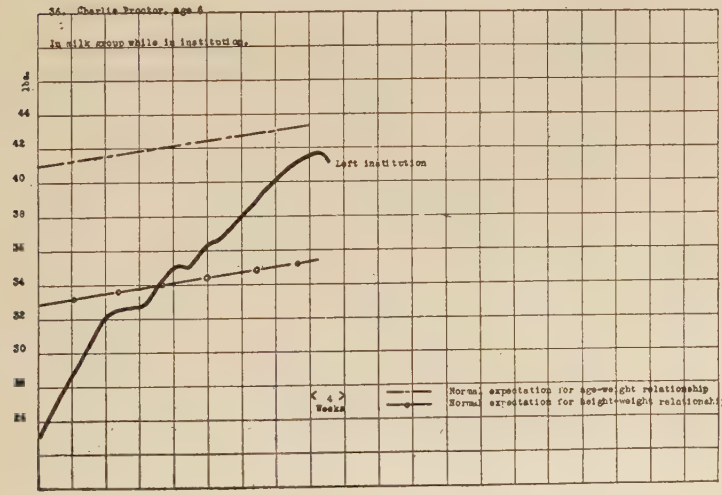


C

CHART 19—*c*. Original check group.—Received institutional diet plus supplementary feeding, and gained. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve.

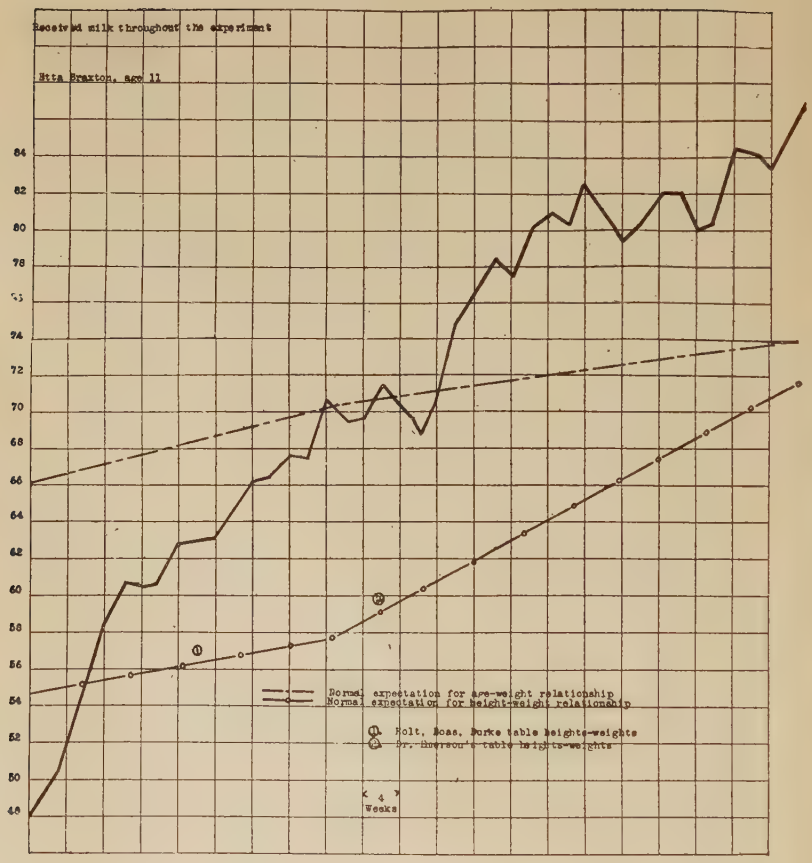

Helen Powell, age 10
Breast bone slightly depressed
Received milk throughout the experiment
Normal expectation for age-weight relationship
Normal expectation for height-weight relationship
4 Weeks

a


34. Charlie Proctor, age 6
In milk group while in institution.
Left institution
Normal expectation for age-weight relationship
Normal expectation for height-weight relationship
4 Weeks

b

CHART 20—*a-b*. Original milk group.—Received milk throughout experiment and gained remarkably. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve.



C

CHART 21—*C*. Original milk group.—Received milk throughout experiment and gained remarkably. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve.

in this institution represented a somewhat exaggerated picture of what exists almost everywhere throughout the country among children in private homes. The results of our study, supplementing as they do the extensive data of animal experimentation on diet, fully justify the concern of those who have been active within recent years in making surveys of the physical condition of school and pre-school children in many cities and in a few country districts. Improvement of the dietary is of the greatest significance for the welfare of a large percentage of American children of the present generation. Leete (1921) summarized the statistics of examination of a large part of the twenty million school children in the United States. She found one per cent to be mentally defective, 5 per cent to be tuberculous or had been in the past, 25 per cent had defective sight, 15 to 25 per cent had diseased tonsils or adenoids, 10 to 20 per cent had deformed feet, 50 to 75 per cent had defective teeth and 15 to 25 per cent were suffering from malnutrition.

Terman (1914) stated that about 14,000,000 of the 20,000,000 school children in the United States were handicapped by some kind of physical defect, and that not far from 2,000,000 were suffering from a grave form of malnutrition. Ten million are said to have enough defective teeth to seriously interfere with health. That the conditions are fully as bad as these figures indicate was borne out by the results of medical examination of young men for the draft in the great war.

Climate as a Factor Influencing Nutrition.—Huntington (1915) has discussed climate as being one of the most important factors in determining the development of a highly progressive race or nation. His studies show that wherever the climate has a favorable range of temperature change from day to night, and is characterized by storminess of the cyclonic type, the people show energy and aggressiveness. As a result of his studies he concludes that a mean temperature of 64° F, a mean humidity of about 80 per cent and frequent changes of temperature are the most desirable conditions for physical work. Statistical evidence is offered to support the theory that only people who live under conditions of relative storminess become industrious, capable and successful.

Japan in respect to storminess ranks as one of the most stimulating regions of the world. It was pointed out in a preceding chapter that the children born of Japanese parents in California

were larger and better developed physically than the children born in Japan. It seems remarkable that a very favorable climate which all would agree Japan has should be less favorable to the growth of its children than a less stimulating climate as found in parts of California. It seems almost certain that a dietary factor is likewise operating to produce the results observed.

Achievements of Peoples of Canada and of the Bahamas.—Although climate is undoubtedly a factor of great importance in determining well-being and consequent achievement, geographers have ignored facts which modern research has brought to light in the field of nutrition which should find a place in any discussion of human progress. A contrast made by Huntington is that of the virility of the Canadians and the improvidence and laziness of the Bahamans. He points out that at the time of the Revolution certain loyalists in the northern states preferred to emigrate to Canada rather than to live under the Stars and Stripes. Other loyalists in the south betook themselves to the inviting climate of the Bahamas for a like reason. It is agreed that their ancestors were equally capable and aggressive although of very different mental types. He emphasizes that the descendants of these Canadians at the present time are industrious, educated and progressive people. Those of the Bahamans are indolent and unprogressive and he believes they have suffered retrogression since their settlement in the islands. The explanation offered for this great difference in quality in the two groups to-day is that the climate of lower Canada is a highly stimulating one, whereas that of the Bahamas is too mild and uniform. It is not permissible to attribute the differences observed between these two peoples solely to climate, for their diet has been so different that there is good reason to suspect that the attitude toward work and mental effort in the two groups is in no small measure due to the way they feel. The Bahamas produce tamarinds, oranges, lemons, olives, limes, citrons, pineapples, pomegranates, figs, sapodillas, bananas, melons, yams, potatoes, cucumbers, cassiva, sugar, maize and peas as the chief articles of food. The breadstuffs and meat are imported from the United States. According to the results of animal experiments this list of foods would not form a satisfactory diet. The people of lower Canada have taken a diet which contained rather liberal amounts of milk, butter, cream and cheese, in addition to cereals, legume seeds, tubers

and meats. People will feel very different on these two types of diets.

Stimulating Climates Not Promoting Health in Man.—If climate were responsible for health to the degree to which geographers believe, how are we to account for the amount of sickness, early aging and physical inefficiency seen in the most favorable climatic areas such as the northeastern quarter of the United States and England, and the fine physical development of the Arabs and the pastoral tribes of Asia, whose climate is less stimulating? It has been pointed out that the teeth of the Americans and English of the present generation are indescribably bad even where the climate is said to be best adapted to health and to the promotion of physical and mental activity. What stands in the way of the progress of Asiatic and African peoples which has kept them far behind the Europeans and Americans in advancing learning? It seems plausible to regard the failure of certain peoples to progress as primarily a geographic one quite as much as climatic. The nature of the land and the amount of rainfall determine the character and vigor of its vegetation, and indirectly the character of the diet. Climate determines the kind of agriculture which is profitable and therefore the most satisfactory mode of life of the people of any region.

Health of American Pioneers.—Poor physical condition is by no means restricted to recent years in this country, nor is it necessarily due to modern conditions of city living. Many will recall how our grandmothers insisted upon the frequent treatment with sulphur and molasses, the periodical dosing with bitters, and the efficacy of sassafras tea in the spring as a means of "thinning the blood," which was supposed to have become impure and "thick" during the winter. Among the pioneer ancestors the idea was generally accepted that there was need for a spring medicine of some kind. There can be little doubt that this belief rested on common experience. We know that in the early settlement of many of the states the people suffered great hardships. With little capital and no food reserve their winter diet was generally very simple and monotonous. There is good reason to believe that it was chemically unsatisfactory for the maintenance of health. After a period of several months during each succeeding winter they felt "run down." The almanacs, which were circulated by patent medicine concerns, discussed

the prevalence of "that tired feeling" in the spring. They convinced people that they needed a course of treatment with sarsaparilla or a "spring tonic" or other "blood purifier." The use of these tonics was so universal that it established the belief that winter was an unhealthful season.

If one now investigates the same communities where a few years ago this condition was regularly observed, one finds well equipped farms, well supplied with dairy herds, orchards and all the signs of modern prosperity. There is much less pronounced difference between the winter and summer diet, due to the abundance of canned fruits and vegetables put up at home or procurable from the stores. More cabbage, turnips, apples, celery, etc., are regularly provided for the winter season. These changes have caused the disappearance of the "tired feeling" in the spring, or better, at the end of winter and consequently the need for spring medicines.

The vendor of spring medicines had a powerful ally in the spring diet of his customers. With the coming of warm weather various "greens" were available and were eagerly consumed. Sorrel pies, wild onions and dandelions grew in abundance on the prairies and were added promptly to the food supply. The cows, due to their starved condition, had been dry all winter, became fresh and milk was available. The few hens in the barnyard added worms, insects and tender grass to their diet and began to lay eggs. The garden furnished fresh vegetables in abundance which were eaten with a relish which can be appreciated only by one who has for a period been semi-starved. The tired feeling disappeared about this time, but the regular spring remedies had been taken and the beneficial results of better diet were wrongly attributed to the efficacy of drugs.

Ehrstrom of Finland (1924), in discussing spring tiredness and vitamin deficiency, states: "We are well in the autumn after the light and open air in the summer and we feel unwell in the spring after the darkness of the winter." Possibly the absence of sufficient vitamin C in the winter diet in Finland may have contributed to deterioration of health in winter.

The Most Satisfactory Type of Diet.—In concluding this chapter it may be well to epitomize the system of living by means of which we and our descendants may recover the physical prestige we are rapidly losing owing to changed conditions, especially with respect to our food habits. The system of diet which can be confidently recommended with assurances that it

will go a long way toward improving the physical fitness of the nation is a very simple one, and its daily practice involves no great self-denial. It involves the borrowing of the best elements from those several systems of diet which have been thoroughly tested in human experience and have been found successful.

The first and most important principle is the extension of the use of dairy products. Instead of the present consumption of half a pint of milk a day there should be about a quart per capita. This is a feature of the diet of all the pastoral peoples of the past and present which has made them superior in physical perfection to all other peoples. The second principle to be kept in mind is that there are dietary properties in the leafy vegetables which are unique among foods of vegetable origin. These have been the "protective foods" for many of the Asiatic peoples. Their consumption in liberal quantities as regular constituents of the diet serves not only to provide the body with valuable nutrients it cannot secure in adequate amounts from milled cereals, tubers and muscle meats, but also serves to maintain the intestinal tract in a hygienic condition through promoting elimination. Milk likewise serves, through its encouragement of the growth of lactic acid-producing organisms (which cause souring of milk), to bring about the disappearance from the intestine of those types of bacteria causing putrefactive decomposition of the food residues with the production of substances which are a physiological abomination. This principle, first enunciated by Metchnikoff, has been shown by bacteriological methods to be sound. Milk has dietary properties which the famous bacteriologist did not discern, and which make it the one food for which there is no effective substitute.

It is essential to keep in mind the third principle of great importance in nutrition, viz., that of taking daily a certain amount of raw vegetable food for the specific purpose of providing the body with a sufficient amount of the anti-scorbutic substance. If these simple principles are adhered to the main features of an adequate diet will be fulfilled, and the remainder of the food supply may safely be derived from any of our ordinary milled cereal products, tubers, root vegetables, sugar and meats. Infants and children can be greatly safeguarded in their skeletal development by providing them at regular intervals with a suitable amount of cod liver oil and by affording an opportunity for outdoor exercise in sunlight.

It would be beyond the scope of the present discussion of nutrition to discuss in an adequate way the details of putting into effect in the home the ideal system of diet. We have treated this subject elsewhere from the point of view of the housewife (*American Home Diet,* 1920).

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